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Green Tea Polyphenol Increases Ap1 Factor-Dependent Human Involucrin (HINV) Gene Expression in Normal Human Keratinocytes

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(–)-Epigallocatechin-3-gallate (EGCG) is an important bioactive constituent of green tea that efficiently reduces epidermal cancer cell proliferation. This inhibition is associated with a reduction in activator protein 1 (AP 1) transcription factor level and activity. However, the effect of this candidate therapeutic agent on normal keratinocyte function has not been extensively explored. Our present studies suggest that EGCG is a potent regulator of normal keratinocyte function. To understand the mechanism of action, we studied the effects of EGCG on AP1 factor activity, and expression of the AP1-factor regulated human involucrin (hINV) gene. hINV is an established, well-studied marker of human epidermal keratinocyte terminal differentiation (Welter *et al, JBC* 270:12614, 1995; Crish *et al, JBC* 273:30460, 1998; Efimova *et al, JBC* 275:1601, 2000). hINV promoter activity is induced in a concentration-dependent manner at 5, 10, 15 and 20 mg per ml of EGCG. An 8-fold increase in activity is observed at 20 mg per ml. This response appears to be physiologic, as a parallel increase in endogenous hINV gene expression is observed. The activation is comparable to that observed for phorbol ester, a known keratinocyte differentiating agent. Mutation of a functionally important AP1 site, AP1-1, located within the hINV gene promoter proximal regulatory region (PRR), eliminates this regulation, suggesting that the response is mediated via an AP1 transcription factor-dependent mechanism. Fra-1, Fra-2, fosB, junB, junD, c-jun and c-fos levels are increased by EGCG treatment. Moreover, AP1 factor binding to the AP1-1 site is increased by EGCG and this complex contains Fra-1 and junD. Thus, our results indicate that EGCG markedly increases AP1 signaling, and differentiation-associated gene expression in normal keratinocytes. This is in sharp contrast to previous reports in transformed keratinocytes, and indicate that the mechanism of EGCG action is markedly different in normal vs. immortalized/transformed keratinocytes.

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The Human Involucrin Gene Contains Spatially Distinct Regulatory Elements that Regulate Expression During Early Versus Late Epidermal Differentiation

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Human involucrin (hINV) is a keratinocyte protein that is expressed in the suprabasal compartment of the epidermis and other stratifying surface epithelia. Involucrin gene expression is initiated early in the differentiation process and is maintained until just prior to terminal cell death. The distal regulatory region (DRR) is a segment of the hINV promoter (nucleotides –2473/–1953) that accurately recapitulates the normal pattern of suprabasal (spinous and granular layer) expression in transgenic mouse epithelia. To identify sequences that mediate expression at specific stages of differentiation, we divided the DRR into two segments, a 376 nucleotide upstream region (DRR-2473/–2100) and a 147 nucleotide downstream region (DRR-2100/–1953), and evaluated the ability of these sequences to drive expression in transgenic mice. The DRR-2473/–2100 segment drives expression at a level comparable to that observed for the DRR, but expression is restricted to the upper granular layers (i.e. no spinous layer expression). In contrast, the DRR-2100/–1953 segment does not drive expression. However, reassembling the DRR restores the complete range of expression. These results suggest that two distinct, spatially separate elements are required to specify the complete differentiation-dependent program of involucrin gene expression. To identify specific transcription factor binding sites involved in this region, we mutated an activator protein-1 binding site, AP1-5, located within DRR-2473/–2100 segment. This site binds AP1 transcription factors present in mouse epidermal extracts, and its mutation eliminates appropriate hINV expression. This result suggests that a multiprotein complex that forms over multiple, spatially separate, regulatory elements is required for appropriate expression of the hINV gene, and that AP1 factors participate as components of this complex.

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Regulation of Human Involucrin Gene Expression by Serine/Threonine Protein Phosphatase Inhibitor Okadaic Acid

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Involucrin is a component of the keratinocyte cornified envelope that is an important marker of keratinocyte terminal differentiation. Okadaic acid (OA) is a potent and specific inhibitor of serine/threonine protein phosphatases PP2A and PP1. The role of okadaic acid-sensitive phosphatases in epidermal differentiation has not been elucidated. In the present study, we examine the effect of this agent on involucrin gene expression. We show that OA enhances human involucrin (hINV) promoter activity in cultured human epidermal keratinocytes, and potentiates phorbol ester-dependent activation of the promoter. The endogenous hINV protein level is also increased by OA treatment, suggesting that inhibition of OA-sensitive phosphatases induces keratinocyte differentiation. Regulation of hINV promoter activity by OA requires the AP1-1 and C/EBP binding sites within the hINV promoter proximal regulatory region (PRR), and activation of hINV gene expression is associated with an OA-dependent increase in both AP1 and C/EBP DNA binding activity, respectively, at the hINV promoter AP1-1 and C/EBP sites. In addition, OA increases the expression of the AP1 transcription family members c-Jun, JunB, JunD, Fra-1 and Fra-2, and alters their phosphorylation state. This increase does not require protein kinase c, as pretreatment with PKC-specific inhibitor, bis-indolylmaleimide, does not block the OA-associated increase in AP1 protein level. C/EBPα and C/EBPβ level are also increased, and their phosphorylation state is altered by OA in a time- and a concentration-dependent manner. These changes are associated with an OA-dependent increase in p38 and JNK activity. Thus, OA appears to increase hINV gene expression via a mechanism whereby the p38 and JNK MAPKs increase AP1 and C/EBP factor levels and DNA binding.

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AP-1 and Sp1 Cooperatively Regulate Differentiation-Specific Expression of the Mouse Loricrin Gene Via Direct Association

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We have previously shown that an AP-1 element in the proximal promoter of the mouse loricrin gene is necessary but not sufficient for expression *in vivo* in transgenic mice. To identify additional regulatory elements required for expression of the loricrin gene, we performed deletion and mutation analysis of the promoter region. Deletion or mutation of a 8-bp sequence (GGGAGGAG), which is located 14 bp upstream of the AP-1 element, resulted in a significant decrease in promoter activity to a level similar to that observed by disruption of the AP-1 element. Electrophoretic mobility shift assays and supershift assays with specific antibodies showed that Sp1 and Sp3 specifically bound to this sequence. Overexpression of Sp1 in cultured keratinocytes resulted in a 12-fold enhancement of the promoter activity, however, Sp3 did not increase promoter activity. Immunoblotting studies using nuclear and whole cell extracts from undifferentiated or differentiated keratinocytes indicated that Sp1 translocated to nucleus in differentiated keratinocytes, potentially inducing differentiation-specific expression of the loricrin gene. Further support for this hypothesis was obtained by cotransfection experiments which demonstrated that c-fos and Sp1 synergistically activated loricrin promoter activity. Interestingly, jun family members (c-jun, JunB, JunD) suppressed the positive effect of c-fos on the loricrin promoter. Site-specific mutagenesis of either the AP-1 and/or the Sp1 element indicated that both elements were essential and acted synergistically for differenciation-specific expression of the loricrin gene. These data suggested functional cooperation between AP-1 and Sp1, therefore we examined the physical interaction between thees two factors. *In vitro* binding by means of a glutathione S-transferase pull down assay showed that both c-jun and c-fos could directly associate with both Sp1 and Sp3. Taken together, these data provide evidence for both physical and functional interactions between AP-1 and Sp1 during differentiation-specific expression of the loricrin gene.

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Different Properties of Three Isoforms of Transcription Factor AP-2 in Human Keratinocytes

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Transcription factor AP-2/promoter system is essential for the complex keratinocyte biology mediated by inflammatory cytokines, as we demonstrated previously (JID 113:600–6, 1999). Three isoforms, AP-2α, β, and γ, share a high homologous structure, but their functions are considered to be different. We therefore studied the implication of each AP-2 isoform in the keratinocyte proliferation, differentiation, and carcinogenesis. Serial skin sections from different sources, normal, psoriasis, and squamous cell carcinoma (SCC), were examined for AP-2 immunoreactivty. AP-2α was present only in the nuclei of normal basal keratinocytes, but significantly increased in the lesional keratinocytes of both diseases. AP-2β was completely absent in all samples, whereas AP-2γ was homogeneously observed throughout the epidermis in normal and psoriatic skin as well as the SCC lesion. There was a strong correlation in the expression pattern between AP-2 isoforms and major keratinocyte markers such as keratin K1, K14, transglutaminase (TGase) I, and epidermal growth factor receptor (EGFR). Furthermore, *in vitro* DNA binding assay revealed the isoform-specific gene activation. AP-2α was highly accessible with the promoter fragments of K14 and EGFR rather than those of K1 and TGase I, all of which are critical regions for the AP-2-dependent gene activation. In contrast, AP-2γ bound to these four promoter fragments with similar affinities, but AP-2β did not despite the binding with AP2 consensus oligonucleotide. These results suggest that three AP-2 isoforms perform unique properties in the spatial and temporal expression of keratinocyte-related genes, thereby maintaining the epidermal homeostasis. Disruption of the epidermal AP-2 balance may lead to the establishment of hyperproliferative and neoplastic phenotypes, such as psoriasis and SCC.

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Transcriptional Regulation of Human Papillomaviruses by Interferon Regulatory Factor-1

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Human papillomaviruses (HPVs) are frequently associated with various lesions of the squamous epithelium. Cell-mediated immune responses against HPV infection result in release of various cytokines that in turn could regulate HPV transcription. Interferon regulatory factor-1 (IRF-1) is induced by several cytokines, and it activates transcription of target genes by binding to a specific sequence in their promoter region. Here we describe the properties of an Interferon Responsive Element (HIRE) in close proximity to the TATA box of HPV type 16. This site resembles the consensus IRF-binding element (IRF-E) or interferon-stimulated response element (ISRE) and overlaps viral E2 binding sites. HPV16 HIRE-1 binds IRF-1 protein in an inducible manner. Mutational analyses revealed the importance of crucial nucleotides in this binding. In a reporter system we demonstrated that HIRE-1 stimulates transcription in response to IRF-1 from both homologous and heterologous promoters in a dose-dependent manner. We also analyzed HIREs of some known mucosal HPV types by gel-shift assay and showed that some high-risk, but not low-risk, mucosal types also bind IRF-1. We can assume that transcription of those HPV types is regulated via HIRE in a fashion similar to that of HPV16. These results might provide important insights regarding the immune responses against HPVs.

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SMaRT Repair of Collagen 17A1 mRNA: Semi-Quantitytive PCR Analysis of *cis*- vs. *trans*-splicing

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Spliceosome mediated RNA *trans*-splicing (SMaRT(tm)) can be used to correct mutant premessenger RNA (premRNA). Recently we demonstrated the repair of a COL17A1 mini-gene premRNA harbouring the recessive mutation 4003delTC located on exon 52 causing generalized atrophic benign epidermolysis bullosa (GABEB). RNA and protein repair by pretherapeutic molecules (PTM's) was shown using a LacZ model-system. To further define the requirements for a gene therapy approach we cotransfected 293T cells and a GABEB cell line with plasmids expressing target (a COL 17A1 mini-gene with the mutation) and PTM6 consisting of a *trans*-splicing domain followed by the correct cDNA sequence spanning exons 52–56 of COL17A1. Trans-spliced mRNA was detected by RT-PCR reactions using gene-specific primers. To determine the percentage of *cis*- vs. *trans*-splicing, PTMs with high *trans*-splicing efficiency were selected using the LacZ repair model. To measure *trans*-splicing efficiency a semiquantitative PCR was performed, using Roche LightCycler and SYBR green. The results showed *trans*-splicing efficiencies up to 6.5% of *cis*-spliced mRNA. This efficiency is low but can be increased by modifiying PTMs and transfection procedures. As shown in the context of the cystic fibrosis gene even 6% of corrected protein might be sufficient for phenotypic correction.

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ICAM-1 Expression in Skin Cells is Dependent on Chromatin Remodeling

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ICAM-1 plays a critical role in cutaneous inflammation. Multiple cytokine-induced, ICAM-1-specific regulatory element and transcription factor interactions have been described in cutaneous cells. However, little is known about modifications of higher order chromatin structure in regions of regulatory elements of the ICAM-1 gene that may be critical to its regulated expression. Histone acetylation causes relaxation of chromatin structure around regulatory elements, making them accessible to activated transcription factors in a promoter-specific fashion. Specific histone acetylases, such as CBP/p300, can act as transcriptional coactivators for STAT1 and NF-κB, transcription factors that are critical to IFNγ- and TNFα-induction of ICAM-1. We asked if IFNγ- and TNFα- induction of ICAM-1 was dependent upon histone acetylation-mediated chromatin remodeling and recruitment of CBP/p300. Acetylated chromatin immunoprecipitation assays, using antiacetyl-histone antibodies to precipitate crosslinked histone-DNA complexes, demonstrated that the IFNγ-response region of the ICAM-1 gene could be PCR-amplified from genomic DNA of IFNγ-treated but not untreated keratinocytes, indicating that acetylation of histones surrounding the IFNγ-response region occurred temporally with IFNγ treatment. Additionally, basal uninduced histone acetylation is normally countered by nuclear deacetylases, keeping DNA regulatory element accessibility to a minimum. We observed that the deacetylase inhibitor, sodium butyrate, induced de novo ICAM-1 mRNA and protein in keratinocytes, further supporting the role of histone acetylation in the regulated expression of ICAM-1. Finally, expression of adenoviral E1A protein, which inhibits the activity of CBP/p300, inhibited the induction of ICAM-1 by TNFα, while mutated E1A had no such effect. These data indicate that induction of ICAM-1 expression in skin cells is dependent upon histone-acetylation-mediated chromatin remodeling and the recruitment of active CBP/p300 acetylases.

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From Enhancesome to Repressosome: Molecular Antagonism Between Corticosteroids and Proinflammatory Cytokines/Growth Factors

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We have shown that glucocorticoids (GCs) suppress, while cytokines/growth factors (TNFα, IL-1 and EGF) induce the expression of keratins K6 and K16. To understand the molecular basis of this antagonism we tested K6/K16 expression during simultaneous presence of GCs and EGF in cotransfection experiments. We found that GCs inhibit the induction of K6/K16 by EGF. We believe GCs antagonize EGF/TNFα on K6/K16 promoters by breaking their enhancesome, a complex structure consisting of an assembly of transcription factors, and converting it to repressosome. To determine the *in vivo* relevance of this switch, we used an organ culture model in which all of the above K6, K16, GCs, TNFα, IL-1 and EGF play important biological roles. We induced K6 expression by making 3 mm wounds and stained sections with a K6 specific antibody. K6 induction, strong at the wound edge, decreased with the distance and was strongly inhibited by topical GCs. This is similar to the findings from cotransfection experiments. In addition, K6 expression induced by application of the EGF, rather than wounding, was completely inhibited by topical GCs in organ cultures. Furthermore, EGF and TNFα treated wounds heal faster than untreated skin, whereas topical GCs inhibit the healing process. When simultaneously present, GCs inhibited healing of the wounds treated with EGF. Since K6/K16, as cytoskeletal proteins, play a role in keratinocyte migration GCs inhibition of their expression and antagonism of EGF/TNFα effects may affect keratinocyte migration. Using a monolayer scratch assay method, we found that EGF and TNFα stimulated, whereas GCs strongly inhibited keratinocyte migration. Importantly, when simultaneously present, GCs inhibited EGF/TNFα effects and keratinocytes did not migrate. We conclude that the switch from enhencasome to repressosome by GCs converts the induction of K6/K16 to repression, which leads to blocking of keratinocyte migration and inhibition of wound healing.

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Human Homeobox HOXA7 Regulates Keratinocyte Transglutaminase Type 1 and Inhibits Differentiation

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Keratinocyte proliferation and differentiation result from expression of specific groups of genes regulated by unique combinations of transcription factors. In order to better understand these regulatory processes, we studied homeobox transcription factor HOXA7 expression, and its regulation of differentiation-specific keratinocyte genes. We isolated HOXA7 from keratinocytes through binding to a differentiation-dependent viral enhancer, and analyzed its effect on coregulated endogenous keratinocyte genes, primarily transglutaminase 1. Induction of keratinocyte differentiation with phorbol ester markedly decreased HOXA7 message, and increased transglutaminase 1 message, in a protein kinase C-dependent manner. Raising the extracellular calcium concentration also repressed HOXA7 and activated TGM1 expression, whereas EGF receptor activation stimulated HOXA7 expression. HOXA7 overexpression repressed transglutaminase 1-reporter activity, and attenuated the transglutaminase 1 induction by phorbol ester, indicating that HOXA7 expression is inversely related to keratinocyte differentiation and transglutaminase 1 expression. Antisense HOXA7 expression activated transglutaminase 1, involucrin, and keratin 10 message and protein levels, demonstrating that endogenous HOXA7 down-regulates multiple differentiation-specific keratinocyte genes. HOX genes function in groups, and we found that HOXA5 too was down-regulated by phorbol ester. These results provide the first example of protein kinase C-mediated homeobox gene regulation in keratinocytes, and new evidence that HOXA7, potentially in conjunction with HOXA5, transrepresses differentiation-specific genes during keratinocyte proliferation, that are then released from inhibition in response to differentiation signals.

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Vitamin D Regulations of Keratinocyte Proliferation and Differentiation Involves the Transcription Coactivator DRIP complex

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The proliferation and differentiation of epidermal keratinocytes involve the sequential transcription regulation of genes critical for this process. Vitamin D (1,25(OH)2D3) and calcium modulate this transcription machinery. The transformation of keratinocytes to squamous cell carcinoma is characterized by impairment of this regulatory system. In order to investigate the molecular mechanism of transcription regulation through vitamin D receptor (VDR), we sought coactivators that have been implicated in transcription regulations by VDR in other cells. We identified coactivators that interact with VDR using an affinity matrix linked to GST-VDR. This enabled the purification of protein complex from nuclear extract of keratinocytes. The complex from proliferating keratinocytes was similar to DRIP complex (vitamin D receptor interacting proteins) previously purified from Namalwa B and Hela cells. This complex contained p205 found in previous studies to directly bind to VDR. In contrast, keratinocyte induced to differentiate by calcium had a different profile with decreased expression of p205, 130, 92, and 77. The squamous carcinoma cells (SCC12B2, SCC4) showed a pattern similar to proliferating keratinocytes. The mRNA of p205 was expressed in proliferating keratinocytes and SCC lines, but was decreased by calcium in normal keratinocytes. These results suggest that the DRIP complex plays a role in vitamin D regulation in keratinocyte proliferation and differentiation.

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Estradiol Stimulates Retinoid Receptor Expression and Potentiates Retinoid Response in Dermal Fibroblasts as Measured by Increased Cellular Retinoic Acid Binding Protein-2 Expression

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Retinoic acid and other retinoids induce the expression of cellular retinoic acid binding protein 2 (CRABP-2) in human dermal fibroblasts. CRABP-2 response in fibroblasts has been suggested to be a reproducible predictor of retinoid bioactivity in human skin. In mouse cervical epithelial cells and estrogen receptor-positive breast cancer cells, estradiol induces retinoid receptor expression. Since human skin cells respond to retinoids and contain estrogen receptors, we evaluated the effects of estradiol on retinoid receptor and CRABP-2 expression in human skin fibroblasts. 1–10 nM estradiol stimulated the expression of RXR-α and RAR-γ by 3–4 fold in fibroblasts as measured by Western Blots. The effect was maximal at 4 h of treatment with estradiol. Retinoic acid, retinol or retinyl esters (nM to uM levels) stimulated the CRABP-2 expression of fibroblasts by 3–5 fold in 48 h. Pretreatment with estradiol potentiated the retinoid effects on fibroblasts CRABP-2 expression. 4 h of pretreatment with 1–10 nM estradiol increased the fibroblast CRABP-2 response to retinoids to 10–30 fold, suggesting a synergy between estradiol and retinoids in fibroblasts. From these studies we conclude that estradiol stimulates retinoid action in skin fibroblasts by increasing retinoid receptor expression.

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Bexarotene (Targretin(r)) Regulates Retinoid Receptor and TIG-3 Expression in Cutaneous T-Cell Lymphoma Lines But Does Not Induce Apoptosis

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Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of extra-nodal, non-Hodgkin's lymphomas in which malignant T-lymphocytes present as skin lesions. Retinoids have long been used alone and in combination with other therapy for CTCL. Bexarotene (Targretin(r)) is a novel synthetic retinoid X receptor (RXR) selective retinoid effective systemically for treating skin manifestations in all stages of CTCL and topically in early stages. Bexarotene inhibits cell growth and causes apoptosis in some leukemic and epithelial cell lines. However, its mechanism of action in CTCL is unclear. To determine whether bexarotene has direct effects on cell proliferation, apoptosis, and modulation of gene expression, CellTiter 96(r) AQ Cell Proliferation Assay, Flow cytometry, Western blotting and QT-RT-PCR were employed to study in well-established CTCL cell lines (MJ, Hut 78 and HH). Over 24–72 h, bexarotene at 0.1–10 μM inhibited cell growth by less than 10% in dose-independent manner, and did not induce apoptosis as determined by Annexin V binding, sub-G1 populations and apoptotic proteins (Bcl-2 and Fas ligand). All three lines expressed α, β, γ isoforms of RARs and RXRs. Bexarotene at 1 μM down-regulated expression of RXR-a protein ($p < 0.05$, $n = 6$) and mRNA ($p < 0.05$, $n = 6$), and up-regulated expression of RAR selective retinoid inducible tumor suppressor, Tazarotene Induced Gene-3 (TIG-3) mRNA ($p < 0.05$, $n = 6$) in Hut 78 and HH lines at 24–72 h compared to controls. In summary, although bexarotene improves the skin manifestations of CTCL in patients, it does not appear to induce apoptosis directly *in vitro* in CTCL cell lines. Bexarotene down-regulates its cognate receptor RXR-a, and may work through inducing expression of TIG-3. *In vivo*, therapeutic effects of bexarotene may also involve indirect mechanisms affecting the survival of T-cells in skin.

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Mutations of Tazarotene-Inducible Gene-3 (TIG-3) Occur in Human Squamous Cell Carcinoma Lines

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Tazarotene-inducible gene-3 (TIG-3) cloned from treated keratinocytes is homologous to a class II tumor suppressor, H-rev 107, and maps to chromosome 11q23, a site of loss of heterozygosity in several malignancies. We previously reported that TIG-3 mRNA and protein are significantly decreased relative to normal skin in psoriasis lesions and in basal and squamous carcinomas [*Clin Cancer Res* 6:3249–3259, 2000]. TIG-3 inhibits growth and is upregulated in cell lines that are inhibited by retinoid treatment, thus we have postulated that TIG-3 may function as a RAR inducible tumor suppressor [*PNAS* 95:14811–14815, 1998]. To study the mechanism(s) responsible for loss of TIG-3 expression, we first determined the structure of the TIG-3 gene using a series of overlapping PCR primers derived from coding sequences to sequence all intron-exon boundaries. The gene was found to consist of four exons of 38 to 317 base pairs, separated by three introns. We next established four exon-specific PCR essays to analyze all coding regions in six human squamous cell carcinoma lines. PCR products from each line were then screened for mutations using SSCP and denaturing high-performance liquid chromatography (DHPLC). Two SCC lines showed shifts of exon-1 on DHLPC that were not detected by SSCP. One lung SCC line (H 460) that lacks both constitutive and inducible TIG-3 expression and is not growth inhibited by retinoid treatment, was found to have a deletion involving both exon-1 and 2. These data suggest that loss of TIG-3 expression in some skin and lung squamous carcinoma lines may result from underlying mutations in the coding regions. Further studies involving the nature of specific mutations and screening of human skin cancer specimens will be necessary to understand the function of the TIG-3 protein and the role of TIG-3 in squamous carcinogenesis and epidermal differentiation.

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Notch1 Functions as an Essential Determinant of Keratinocyte Exit from the Cell Cycle and Entry into Differentiation

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The molecular mechanisms that control the balance between epithelial cell growth and differentiation are likely to involve a complex interplay between classical cell regulatory pathways and development-related signals. Our findings indicate that when primary keratinocytes are induced to differentiate endogenous Notch activity increases, while inhibition of Notch activation suppresses differentiation. Ectopic expression of activated Notch1 causes keratinocyte growth arrest by a p21WAF1/Cip1- dependent mechanism, inducing p21WAF1/Cip1 expression at the transcription level in a RBP-Jk-dependent fashion. Activated Notch1 induces also the early differentiation markers associated with the transition from the basal to intermediate epidermal layers, while suppressing the late markers characteristic of the outermost epidermal layers. In parallel with these effects, enhanced Notch1 activity causes loss of adhesion of keratinocytes to the substratum and down-modulation of integrin β1 and β4 expression. Thus, Notch1 appears to function as a key determinant of the ordered transition between keratinocyte growth and early vs. late stages of differentiation.

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Modulation of mRNA Level of Darier Disease Gene in Cultured Human Keratinocytes by UV and Calcium Concentration and the Effects of Antisense Oligonucleotide on ATP2A2 in Cultured Skin Explant

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Darier Disease (DD) is an autosomal dominantly inherited skin disorder characterized histologically by acantholysis and dyskeratosis of keratinocytes. Although DD was found to be caused by mutations in the ATP2A2 encoding sacro/endoplasmic reticulum calcium ATPase type 2 isoform (SERCA2), the pathomechanism links between gene mutations and the formation of unique histology is still uncertain. In this study, we initially examined the mRNA expression levels of ATP2A2 in cultured normal human keratinocytes using the on-line fluorescence quantitative RT-PCR system (Light Cycler) after UV-B irradiation and by increasing extracellular calcium concentration. We then determined the effects of SERCA inhibitor (Thapsigargin) and sense and antisense oligonucleotides on SERCA2 and SERCA 3 in cultured skin explants. The expression of ATP2A2 mRNA in cultured keratinocytes was suppressed temporarily after UV irradiation. A temporary increase was achieved after switching from a low to high extracellular calcium concentration. The SERCA inhibitor, the antisense oligonucleotide to SERCA2 (but not the sense oligonucleotide to SERCA2) and the antisense oligonucleotide to SERCA3 can induce morphological changes similar to DD in organ cultured normal human skin explant. These results suggest that modulation of ATP2A2 mRNA expression by environmental factors might contribute to the formation of the lesions seen in DD and points to haploinsufficiency as being the pathological mechanism underlying the disease. Because ATP2A2 hemi-knock out mouse did not show any skin manifestations, this organ culture system will most likely have application only as a skin model for DD.

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Wnt-4 is Down-Regulated Early in Wound Healing in the Rat: Elucidating the Role of Morphogens in Tissue Repair Processes

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The Wnt family of morphogens is important in developmental processes such as body axis development, CNS formation and limb/digit growth. Wnt-4 in particular has been detected in wounds and is thought to be important in epithelial–mesenchymal interactions in the skin. Prior studies of Wnt expression in wounds were limited due to the use of degenerate PCR primers. We therefore sought to definitively determine the expression of Wnt family members in wounds, and to determine if the expression of Wnt-4 is altered during the course of skin wound repair. A 6-mm punch was used to create full-thickness wounds on the dorsal skin of 30 rats. Using gene-specific primers, we detected by RT-PCR the expression of 10 different Wnt genes, including Wnt-4, in unwounded skin and wounds. In addition, we utilized nonradioactive RNAse protection assays to measure the relative changes in Wnt-4 and GAPDH expression as a function of healing. GAPDH levels did not vary at any of the time points tested. However, Wnt-4 RNA expression was down-regulated by 60% as early as 12 h postwounding; by day 7 levels had risen to 75% that of unwounded skin and had normalized to levels found in unwounded skin by day 21. This is the first report to show a change in a Wnt family member during wound healing processes. In addition, we have demonstrated a more widespread expression of Wnt family members in skin and wounds than has been previously been reported. These results suggest that the Wnt family of morphogens may be important in the baseline maintenance of skin morphology as well as the restoration of proper form and architecture following skin injury.

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Generation of Inducible NF-κB Subunits: Confirmation of a Primary Growth Inhibitory Role for NF-κB in Epidermal Cells

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The IKK/NF-κB axis has recently been appreciated as a dominant epidermal regulator, with IKK dysfunction established as central in incontinentia pigmenti and constitutive NF-κB activation leading to epidermal growth inhibition. These data suggest that NF-κB may be the primary mediator of the effects of this axis on epidermal growth. The relative importance and physiologic role of NF-κB subunits, however, has recently been questioned as an artifact of constitutive overexpression and a model advanced in which growth inhibition of this axis is mediated by IKK targets other than NF-κB. To resolve this, we have generated inducible mutant estrogen receptor-(ER) fusions of all five NF-κB subunits [p65, p50, p52, c-rel, relB] to define the effects of direct, temporally regulated NF-κB induction. Expression of the NF-κB subunit ER-fusions in primary keratinocytes via retroviral transduction produced expected NF-κB-driven transcription in the following order of potency p65 RelB c-rel p50 p52. NF-κB-ER fusions translocated from cytoplasm to nucleus upon tamoxifen treatment in primary keratinocytes. This induction was specific in that it did not alter transcription directed by other promoter elements, including those driven by AP1. Upon induction, all 5 NF-κB subunits caused rapid keratinocyte growth inhibition with the level of inhibition correlated with the level of transcriptional potency of each subunit. These growth inhibitory effects were blocked by a dominant-negative form of IκB that can physically bind and sequester all 5 NF-κB subunits, indicating a primary role for NF-κB in triggering epidermal cell growth arrest. To study the effects of temporally controlled NF-κB induction *in vivo*, transgenic mice targeting all 5 inducible subunits to epidermis are being generated. These data indicate that direct induction of NF-κB is sufficient to trigger epidermal growth arrest, characterize a full panel of NF-κB subunits subject to exogenous control and indicate that NF-κB subunits exert differential activity in growth inhibition associated with their transcriptional potency in epidermal cells.

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Regulated Induction of Ras and its Effectors in Epithelial Cells

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Ras GTPases, acting via 3 main effector pathways, Raf, PI3K and RalGDS, alter growth and differentiation in many tissues. Ras effects, however, can vary depending on tissue setting and signal strength, with strong constitutive Ras signal inductive of growth arrest in normal cells. In epidermis, Ras is best characterized in neoplasia models, however, these studies rely on a constitutive Ras activation that may not reflect the impact of the spatially controlled, physiologic strengths of Ras activation we have recently identified in normal epidermis. To regulate Ras signal strength and study the range of Ras function in a temporally controlled manner, we generated a panel of tamoxifen (4OHT)-responsive mutant estrogen receptor (ER)-H-Ras fusions, including N-terminal ER additions and myristylated C-terminal fusions; to study relative contributions of Ras downstream effectors, we have also generated ER-fusions to Raf, RalGDS as well as the PI3K target Akt. N-terminal ER-Ras fusions displayed no detectable leakiness and strongly induced Ras activity in response to 4OHT when expressed in primary keratinocytes via retroviral transduction. Addition of high concentrations of 4OHT, while displaying no effect on growth or differentiation of lacZ controls, inducibly suppresses growth in cells expressing ER-Ras as does overexpression of constitutively active Ras; ER-Ras expressing cells without 4OHT proliferate normally. In addition, inducible Ras actively suppresses induction of keratinocyte differentiation markers by calcium. Interestingly, inducible activation of Akt leads to increased differentiation marker expression and growth inhibition suggesting that the impacts of downstream Ras effectors may exert distinct – and sometimes opposing – effects and that non-PI3K pathway Ras effectors may be dominant mediators of Ras effects on cellular differentiation in this setting. Consistent with this, Raf-ER fusions inducibly suppress differentiation. The effects of regulated induction of Ras and its effectors are being characterized in epidermal tissue via generation of mice transgenic. Taken together, these data provide a new approach to regulate the function of Ras and its effectors in order to study Ras pathway impacts on epidermal growth and differentiation.

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Iron Chelators Inhibit VCAM-1 Expression in Human Dermal Microvascular Endothelial Cells

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Vascular cell adhesion molecule (VCAM) 1 gene expression may be coupled to oxidative signals through specific redox-sensitive regulatory pathways. Iron may play a role in generation of reactive oxygen and lipid species that participate in signaling pathways. To investigate the role of iron in VCAM-1 gene expression, human dermal microvascular endothelial cells (HDMEC) were stimulated with TNF in the presence or absence of iron chelators and examined for induced expression of VCAM-1 protein and mRNA by ELISA and real-time PCR, respectively. Both dipyridyl (DP) and desferrioxamine (DFO) inhibited VCAM-1 protein and mRNA induction in a dose- and time-dependent manner. DP also inhibited the induction of ICAM-1 and E-selectin, but inhibition required higher doses than those effective in blocking VCAM-1 induction. In order to rule out a metal chelation-independent antioxidant effect, we examined the effect of DMPO, a nonmetal binding ROS scavenger. The induction of VCAM-1, ICAM-1 and E-selectin was not inhibited by DMPO, implying a direct effect of iron in the expression of these adhesion molecules. Since oxidative signaling has been implicated in NF-κB activation, we utilized electrophoretic mobility shift assays to examine whether DP pretreatment of HDMEC would inhibit TNF-mediated NF-κB activation and translocation. TNF treatment resulted in increased binding of nuclear-derived p50/p65 complexes to an oligonucleotide corresponding to the VCAM-1 kB elements. Pretreatment of HDMEC prior to TNF treatment had no effect on the nuclear localization of these complexes. These data demonstrate that iron chelation inhibits cytokine mediated VCAM-1 protein and mRNA up-regulation but does not affect NF-κB activation or DNA binding.

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MEK7-Dependent Activation of p38α Map Kinase Increases Differntiation-Associated Gene Expression in Keratinocytes

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However, the kinase that functions immediately downstream of MEK7 has not been identified. Our present studies show that constitutively active MEK7 markedly increases p38α activity, but not the activity of the other p38 isoforms (β, δ and γ). This p38α-dependent activation is not accompanied by any change in p38α levels. Pharmacologic inhibitors of p38α activity also inhibit the MEK7-dependent increase. Additional evidence shows that the other major MAPK signaling families, the JNK and ERK kinases, do not participate as MEK7-dependent gene activators. We also demonstrate that this regulation is physiologically important, as the MEK7-associated p38α activity increases hINV promoter activity and expression of the endogenous hINV gene via an AP1 transcription factor-dependent mechanism. The observation that MEK7 regulates gene expression via activation of p38 MAPK signaling is a completely novel finding, as previous reports, in a variety of systems, have assigned JNK as the sole downstream target of MEK7. We conclude that MEK7 regulates hINV gene expression via a novel MAPK regulatory cascade that includes PKC, Ras, MEKK1, MEK7, and p38α.

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MEK6 Regulates Human Involucrin Gene Expression Via a p38α and δ-Dependent Mechanism

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Involucrin (hINV) is an important marker of epidermal keratinocyte differentiation, and hINV gene expression is regulated by signals transmitted via the mitogen-activated protein kinase (MAPK) cascades. A MAPK cascade that includes PKC, Ras, and MEKK1 regulates involucrin (hINV) gene expression in epidermal keratinocytes (Efimova *et al*, *J Biol Chem* 273:24387, 1998; Efimova *et al*, *J Biol Chem* 275:1601, 2000). However, since signal transfer downstream of MEKK1 may involve more than one of the seven MAPK kinases (MEKs), it is important to evaluate the regulatory role of each MEK isoform. In the present study we confirm that MEK6 is expressed in keratinocytes and we evaluate the role of MEK6 in transmitting the MEKK1-dependent signal. We show that constitutively active MEK6 (caMEK6) increases hINV promoter activity and increases endogenous hINV levels, while dominant-negative MEK6 (dnMEK6) decreases expression. The caMEK6-dependent increase in gene expression is inhibited by the p38 MAPK inhibitor, SB203580, and is associated with a marked increase in p38α MAPK activity; JNK and ERK kinases are not activated. In addition, hINV gene expression is inhibited by dominant-negative p38α. caMEK6 also activates p38δ and overexpression of p38δ inhibits the caMEK6-dependent increase in hINV gene expression. These results suggest that MEK6 increases hINV gene expression via a unique mechanism that involves modulating the balance between activation of p38α, which increases gene expression, and p38δ, which decreases gene expression.

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Oligonucleotide-Induced MAP Kinase Signaling Events in Human Keratinocytes

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Oligonucleotides (ODNs) are currently under investigation for therapeutic purposes due to their immune stimulatory effects mediated through CpG sequences. Furthermore, antisense ODNs are used as agents against cancer and viral infections. Because skin gene therapy is a new approach to treat diseases, we started to examine the *in vitro* modulation of the mitogen-activated protein (MAP) kinase signaling pathways and of activator protein (AP) 1 and AP2 transcription factors in normal human keratinocytes (NHKs) exposed to ODNs. Using immunoblot techniques, we found AP2 almost exclusively in the nuclear compartment upon exposure of NHKs to ODNs (100 pM, 12 h). In addition, AP2 protein was decreased when compared to unstimulated or phorbol ester (TPA) treated cells. MAP kinase p38 was induced as soon as after 2 min by ODN exposure and returned to basal levels within 15 min Control stimulation with TPA induced maximum p38 MAP kinase phosphorylation at 15 min Therefore, early and short-termed p38 MAP kinase activation may be responsible for the induction of inflammatory cytokine production in NHKs upon exposure to ODNs.

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Differential Target Gene Induction Mediates Opposing Growth Effects of NF-κB in Keratinocytes and Fibroblasts

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NF-κB influences cellular processes that include inflammation, apoptosis and cell division. In epidermis, NF-κB inhibits both growth and death, in contrast to its effects in other cell types which can include promotion of both processes. In skin, for example, the NF-κB inducer TNF-α is associated with growth inhibition in keratinocytes and growth promotion in dermal fibroblasts. To explore the basis for these differences, we expressed NF-κB in primary keratinocytes and fibroblasts at 95% efficiency via retrovectors. In order to identify NF-κB targets we used high density cDNA microarrays and 2D gel analysis to characterize NF-κB genomic and proteomic targets. Striking differences between keratinocytes and fibroblasts were found in NF-κB induction of growth regulatory genes. In keratinocytes, growth inhibitory genes including p21 and GIF (Growth Inhibitory Factor) were up-regulated by NF-κB, whereas in fibroblasts these genes were not induced. Instead, growth promoting genes including cyclin B1, FGF-2 and FGF-5 were up-regulated. Consistent with this, NF-κB caused growth arrest in keratinocytes while it supported rapid fibroblast proliferation. Overexpression of p21 alone in keratinocytes is sufficient for growth inhibition similar to that caused by NF-κB and, not surprisingly, p21 has the same effect in fibroblasts, indicating that the difference in NF-κB growth effects in the two cell types is determined by differential target gene induction. p53 is known to be involved in p21 up-regulation in response to UV irradiation. To test whether the induction of p21 by NF-κB in keratinocytes is p53-dependent, we transiently transfected NF-κB or its super repressor IκBaM, along with a reporter construct expressing luciferase driven by the p53-binding site of the p21 promoter. No significant difference was observed in p53-directed transcription compared with control, indicating that NF-κB induction of p21 may be p53-independent in keratinocytes. These data suggest that cell type specific responses to NF-κB are mediated by differential target gene induction, that cell specific effects can be mimicked by expressing target genes and that NF-κB growth inhibition in keratinocytes may mediated through p53-independent induction of growth inhibitory targets that include p21Cip1 and GIF.

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Phospholipase C-γ1 is Required for 1,25-Dihydroxyvitamin D-Induced Keratinocyte Diffrentiation

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Keratinocytes produce vitamin D_3 and convert it to the most active form, 1,25-dihydroxyvivtamin D_3 [$1,25(OH)_2D_3$] which in turn regulates keratinocyte proliferation and differentiation. Phospholipase C-γ1 is the most abundant member of the phospholipase C family in keratinocytes and is induced by $1,25(OH)_2D_3$. Therefore, we examined the hypothesis that phospholipase C-γ1 played an essential role in the signaling pathway mediating $1,25(OH)_2D_3$-induced keratinocyte differentiation. Phospholipase C-γ1 expression in human keratinocytes was reduced by transfecting the cells with an antisense PLC-γ1 construct then evaluating the response of the keratinocyte differentiation markers involucrin and transglutaminase to $1,25(OH)_2D_3$. The results showed that $1,25(OH)_2D_3$ induced involucrin and transglutaminase protein and mRNA levels were markedly reduced in keratinocytes transfected by the antisense phospholipase-γ1 construct. Similarly, cotransfection of keratinocytes with an involucrin or transglutaminase promoter construct and the antisense phospholipase C-γ1 construct showed decreased involucrin and transglutaminase promoter activity, respectively, in response to $1,25(OH)_2D_3$. To further investigate the mechanism by which phospholipase-γ1 regulates keratinocyte differentiation, the calcium level in keratinocytes transfected by the antisense phospholipase C-γ1 construct was measured following $1,25(OH)_2D_3$ administration. The increase in keratinocyte intracellular free calcium level following $1,25(OH)_2D_3$ administration was markedly reduced by the transfection of the antisense phospholipase C-γ1 construct. These studies indicate that phospholipase C-γ1 plays a critical role in the signal transduction pathway mediating $1,25(OH)_2D_3$-induced keratinocyte differentiation at least in part by mediating the increase in intracellular calcium mobilization following $1,25(OH)_2D_3$ administration.

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Delayed Dermal Fibroblast Aging and Promotion of a Matrix Synthetic Phenotype with Novel Nitrone-Based Free Radical Trap Molecules

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Innate cutaneous aging progresses as a consequence of organismal aging and results in skin deficiencies such as fine wrinkling, epidermal and dermal atrophy, loss of subcutaneous fat, decreased rates and quality of wound repair, and an increased susceptibility to wounds that fail to heal. The free radical theory of aging proposes that reactive oxygen species cause oxidative damage over the lifetime of the organism and that the accumulated oxidative insults contribute to many of the dysfunctions and pathologies associated with aged skin. Previous studies have implicated oxidative damage in promoting replicative senescence in dermal fibroblasts. Senescent fibroblasts accumulate in the dermis with increased age, remain metabolically active, and switch their gene expression patterns from that which supports the extracellular matrix milieu to one that results in progressive destruction of the tissue (e.g. up-regulation of collagenase-1 and down-regulation of type I collagen). Nitrone-based free radical trap molecules have been shown to increase lifespan and reverse age-associated deficiencies in cognitive function in an experimental mouse model. Furthermore, treatment of human diploid fibroblasts with a molecule from this class (α-phenyl-N-tert-butyl-nitrone, PBN) delays senescence and rejuvenates those close to senescence. The present study was undertaken to determine if CX-412, a novel nitrone-based free radical trap, could prevent dermal fibroblast senescence or reverse the gene expression patterns associated with senescent cells. Human dermal fibroblasts were isolated from neonatal foreskins and aged *in vitro* in the presence of vehicle control or CX-412 (25–500 μM). We found that extended treatment of dermal fibroblasts (4 days to 3 months) with CX-412 resulted in a dose-dependent stimulation of clonal cell growth and 3H-thymidine uptake, significantly improved cell morphology, and a marked reduction in the expression of senescence-associated β-galactosidase activity when compared to vehicle control. Interestingly, cells treated with CX-412 had an extended lifespan of approximately 10 population doublings when compared to cells treated with vehicle alone. CX-412-treated fibroblasts were not immortalized or transformed, however, as no telomerase activity or growth in soft agar was noted. CX-412 also reversibly inhibited the expression of genes associated with senescent fibroblasts. Cells treated with CX-412 chronically (4 days to 3 months) or acutely (0–72 h) demonstrated a significant dose-dependent inhibition of collagenase-1 and enhanced expression of type I collagen mRNA. Thus, treatment of dermal fibroblasts with CX-412 prolongs proliferative lifespan and prevents the expression of senescence-associated proteolytic, destructive genes and induces a matrix synthesis phenotype.

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Up-Regulation of Galanin Gene Expression by Heat Treatment in Human Keratinocytes

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Neuropeptides, that innervate the skin mediate actions important in skin inflammation and wound healing by induction of vasodilatation, plasma extravasation, cytokine production or adhesion molecule expression. Based on our previous observation that the neuropeptide galanin (GAL) is expressed in epithelial cells of the skin (1), we analysed the thermal regulation of GAL gene expression in the human keratinocytes (KC). mRNA expression of GAL and Heat Shock Protein 70 (HSP70) in primary cultured human KC was detected by Northern Blot analysis. GAL-like immunoreactivity was determined by immunohistochemistry and GAL peptide concentration by ELISA. Northern Blot analysis revealed a 100-fold induction of GAL and HSP70 mRNA in KC by heat treatment at 42°C. *In vivo* the GAL-like immunoreactivity in KC of human epidermis is decreased 2 h after heat treatment (75 J per cm^2). After 24 h GAL staining was similar to untreated skin. Furthermore, we detected high amounts of GAL peptide in the supernatant of heat treated human cultured KC and blister fluid of burned human skin. Therefore, we speculate that GAL released by KC might be involved in the pathophysiology of the burn reaction probably via its known effect on vasodilatation and plasma extravasation.

1. Kofler *et al*, *Arch Derm Res* 210:56, 1998

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Identification of Novel TGF-β/Smad Gene Targets in Dermal Fibroblasts Using a Combined cDNA Microarray/Promoter Transactivation Approach

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Despite major advances in the understanding of the intimate mechanisms of transforming growth factor-β (TGF-β) signaling through the Smad pathway, little progress has been made in the identification of direct target genes. In this report, using cDNA microarrays, we have focussed our attention on the characterization of extracellular matrix-related genes rapidly induced by TGF-β in human dermal fibroblasts, and attempted to identify the ones whose up-regulation by TGF-β is Smad-mediated. For a gene to qualify as a direct Smad target, we postulated that it had to meet the following criteria: (1) rapid (30′) and significant (at least 2-fold) elevation of steady-state mRNA levels upon TGF-β stimulation (2) activation of the promoter by both exogenous TGF-β and cotransfected Smad3 expression vector, and (3), up-regulation of promoter activity by TGF-β blocked by both dominant-negative Smad3 and inhibitory Smad7 expression vectors. Using this stringent approach, we have identified COL1A2, COL3A1, COL6A1, COL6A3 and TIMP-1 as definite TGF-β/Smad3 targets. Extrapolation of this approach to other extracellular matrix-related-gene promoters also identified COL1A1 and COL5A2, but not COL6A2, as novel Smad targets. Together, these results represent a major step toward the identification of novel, early induced, Smad-dependent TGF-β target genes in fibroblasts.

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cDNA Microarray Analysis of Gene Expression in Non-Melanoma Skin Cancer

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We have sought to determine gene expression differences between basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) lesions and normal skin at a genome-wide scale. Total RNA was prepared from lesional and site-matched nonlesional skin biopsy samples from patients with biopsy proven BCC or SCC and enriched for mRNA. cDNA was then synthesized, radiolabeled, and hybridized to Atlas Human Cell Interaction Arrays. These arrays allow the simultaneous analysis of expression of 265 genes. Expression was quantitated using a Storm 840 PhosphorImager, and analysis was performed using ImageQuant 1.2 software (Molecular Dynamics). Preliminary results for BCC show that 13 genes exhibited differential expression, which was defined as a two-fold or greater difference in expression between lesional and nonlesional tissue, in at least two patients. Genes of interest include the malignant melanoma metastasis-suppressor gene and the L1 neural cell adhesion molecule precursor, which have been previously implicated in melanoma. Also of note were ezrin, which is in the same gene family as moesin (previously shown to have altered expression in BCC and SCC); and PuF, which has been shown to transactivate the c-myc gene (overexpressed in many tumors of the skin). Preliminary results for SCC show that approximately 20 genes exhibited differential expression. Genes of interest include sonic hedgehog, patched homolog, and smoothened, all of which have previously been implicated in BCC but not SCC. These results are being confirmed with a larger data set and with Affymetrix Human Genome U95A Arrays, which permit the simultaneous analysis of expression of approximately 12 500 sequences. These data on the global patterns of gene expression in BCC and SCC should allow further insights into disease pathogenesis and basic mechanisms of skin oncogenesis.

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Gene Expression Profiling in Inflammatory Skin Disease by cDNA Microarray Analysis

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Inflammatory skin diseases, including psoriasis vulgaris and atopic dermatitis, are chronic, polygenetically determined skin disorders. The expression levels of many genes have been shown to be increased in lesional psoriatic and atopic skin, but in spite of these advances, broad perspectives on the gene expression abnormalities characterizing these disorders have been unavailable. Thus far, functional genomic studies have been of limited scope, only able to elucidate the role of one or a few genes at a time in one system. To this end, we are utilizing the technology of high density oligonucleotide arrays, or so-called ''gene chips'', to systematically and comprehensively document differential gene expression between psoriatic and atopic skin and normal skin at a genome-wide scale. Total RNA was prepared from lesional and site matched nonlesional skin biopsy samples from five patients with stable chronic plaque psoriasis, and from two patients with atopic dermatitis. The RNA was then reverse transcribed and labeled using fluorescent nucleotides, followed by hybridization to Affymetrix Human Genome U95A Arrays. These arrays permit the simultaneous analysis of expression of approximately 12 500 sequences that have been previously characterized in terms of function or disease association. Up- and down-regulated sequences are currently being confirmed and catalogued. Study of global patterns of gene expression in psoriasis and atopic dermatitis form a basis for further insights into the pathogenesis of inflammatory skin disease.

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Analysis of the UVB Response in Primary Human Keratinocytes Using Oligonucleotide Microarrays

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UV light is the most important physical carcinogen in the environment, and the skin is its natural target. UVB irradiation of skin keratinocytes produces characteristic mutations, but also causes the induction or repression of a number of genes. Our aim is to identify the subset of genes whose transcription is altered during the UVB response in human keratinocytes using microarray technology. We have used commercial high density oligonucleotide arrays (HuGeneFL, Affymetrix), capable of detecting transcript levels of over 6000 human genes, to analyze RNAs from primary human keratinocytes irradiated with three different UVB doses (10, 20 and 40 mJ per cm^2) at two time points post irradiation (4 and 24 h). The data analysis shows that approximately 10% of the represented transcripts are affected by UVB irradiation. Approximately 200 genes changed their expression 4 h after irradiation, 30% of which were induced. At 24 h, the number of affected genes was increased to around 550, and the percentage of induced genes also rose to 50%. Using GeneCluster software these genes were further organized into nine discrete groups, according to their pattern of induction or repression at the different times and doses. In addition to finding genes already known to respond to UVB, such as GADD45, cyclooxygenase 2, and members of the matrix metalloprotease and interleukin gene families, we have also identified several genes whose response to UV has not been described. These include transcripts involved in adhesion, angiogenic and proliferative events. Our data demonstrate the complexity of the transcriptional response to UVB light in keratinocytes and supports the use of this kinetic model to undertake a global characterization of UV regulated genes and pathways.

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Characterization of a Complex Interacting Gene Regulation Profile by Microarray Analysis in Human Skin Triggered by Simulated Solar Radiation

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DNA microarray analysis allows establishment of a pattern of gene expression from multiple genes and facilitates an understanding of the complex interactions elicited by selective interventions such as exposure to ultraviolet radiation. In this study, keratome biopsies were taken from exposed and normal skin of five human subjects 32 h following exposure to 4 MED solar simulated radiation (SSR). To minimize interindividual variation, fractions of tissue from the five subjects were pooled prior to RNA extraction. Following microarray hybridization, gene expression was quantified via phosphoimage analysis, normalized to the average density of all the genes on the membrane and corrected for background hybridization using ImageQuaNT^{TM} software. The entire extraction, array procedure, and calculation of the gene changes was repeated five times on the pooled tissues, and the change in each gene's expression from five hybridizations was averaged ($n = 5$). Gene changes two standard deviations either above or below baseline were considered significant and provided an expression profile associated with SSR. Up-regulation of gene expression following SSR was confirmed by RT-PCR analysis for selected genes. Thus, these findings constitute a unique gene expression/regulation profile of human skin under *in vivo* SSR challenge. Study of selective profile modifications as well as specific novel gene changes following *in vivo* interventions may lead to new understandings of complex regulatory pathways and the relative efficacy of interventions.

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Identification of Mutated as well as Differentially Expressed Sequences in a Cutaneous Lymphoma Line by the Novel Ligation Mediated Subtraction (Limes)

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Primary cutaneous T- and B-cell lymphoma have a largely unknown etiology and exhibit considerable variation in clinical presentation, histology, immunophenotype or prognosis. In order to contribute to a better understanding of their biology and etiology, the representational difference analysis (RDA) was used to isolate tumor-associated mutations from a cutaneous lymphoma cell line (My-La). RDA and other subtraction techniques allow to enrich sample-specific sequences by elimination of ubiquitous sequences existing in both, the sample of interest (tester) and the subtraction partner (driver). After application of RDA to genomic DNA, however, repetitive sequences and artificial fusion products of otherwise independent PCR fragments (PCR hybrids) were predominately isolated. Since these products severely interfered with the isolation of tumor-relevant fragments, we developed a considerably more robust and efficient approach, which is termed ligation mediated subtraction (Limes), because the resistance of this novel method to artifact production is mediated by a highly specific and exclusive ligation of oligonucleotides to the ends of perfectly matched tester/tester hybrids. In first applications of Limes, genomic sequences and/or transcripts of genes involved in the regulation of transcription such as the transforming growth factor b stimulated clone 22 related gene (TSC-22R), cell death and cytokine production (caspase-1), or antigen presentation (HLA class II) were found to be absent in My-La. Moreover, mutations influencing the transcription pattern of the affected genes, were identified by a modified Limes protocol. Due to these results, Limes may substitute/supplement other techniques such as RDA or DNA microarray techniques in a variety of different research fields.

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Microarray Analysis of Gene Expression Following Exposure to Skin Irritants

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Understanding of the mechanistic basis of the human skin irritation response is key to the development of relevant *in vitro* test systems for the predictive identification of skin irritation hazards. Recent progress in genomic technologies means that tools for the identification and investigation of important biochemical events in the processes of skin irritation are now available. This work was designed to identify genes for further mechanistic investigation which are regulated in response to skin irritation, following exposure of the EpiDerm(tm) skin model to the known irritant sodium lauryl sulphate (SLS). EpiDerm(tm) cultures were treated in triplicate with a noncytotoxic dose of SLS (0.1 mg per ml, as determined by the MTT assay and histological examination) for 15 min, 30 min, 1 h, 2 h, 3 h, 4 h and 24 h. Total RNA was extracted from pooled EpiDerm(tm) cultures and used to probe Atlas(tm) human arrays (Clontech) covering approximately 3600 genes. Data indicated an up-regulation at early time points (15–30 min) of a number of genes involved in transportation (e.g. the sodium and chloride dependent taurine transporter) and receptors (e.g. ZAP70). The gene encoding the UV excision repair protein and other DNA repair genes (e.g. DNA-directed RNA polymerase II) were up-regulated at 1–3 h along with TGFβ3 and other tumour suppressors (which play a role in cellular development and wound healing). At the later time points of 4–24 h, genes involved in protein translation (e.g. Cathepsin D receptor) and metabolism (e.g. CYP27A) were up-regulated. In addition, a number of genes were down-regulated in response to treatment with SLS, although these followed less of a time dependent pattern. These results indicate the differential regulation of a number of genes in response to treatment with SLS which may provide insights into the molecular events in the response to this irritant.

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Microarray Analysis of Cutaneous Basal Cell Carcinomas

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Background: Basal cell carcinomas (BCC) are the most common cutaneous malignancy. Although linked to UV exposure, the carcinogenic pathway of BCC has not been elucidated. Microarray analysis is a high through-put method of screening thousands of genes simultaneously. We used microarray analysis to identify gene expression differences between BCC and normal keratinocytes. Design: Fresh tissue from 7 cases of cutaneous BCC, including surrounding normal skin, was obtained. Labeled cDNA probes were synthesized (Cy5 for tumor, Cy3 for normal), and applied to microarrays containing approximately 9000 human genes. The microarrays were scanned and analyzed to determine gene expression. BCC genes were considered overexpressed compared to normal epidermal genes when the red to green ratio was greater than 1.8. Results: We identified 9 genes overexpressed in at least 5 of the 7 BCC (compared to corresponding normal tissue). A third of these are known cancer associated genes, including squamous cell carcinoma antigen 1, and S100 calcium binding protein. One EST (expressed sequence tag) was increased in 6 of the 7 BCC. In addition, a subgroup of four closely related tumors was isolated by gene cluster analysis. These tumors over-expressed a group of genes not increased over normal skin in the other BCC samples. Conclusion: Microarray analysis is useful in identifying potential genes involved in BCC carcinogenesis. In addition, different subgroups of BCC may be revealed, which may differ clinically as well as genetically.

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Constitutive Expression and Distribution of Cytochrome P450 Isozymes in Normal Human Skin

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Cytochrome P450 (CYP) isozymes play a major role in drug metabolism. The expression and distribution of CYPs in the skin would be helpful in understanding the cutaneous drug metabolism. The aim of this study is to examine the constitutive mRNA expression and distribution of CYP isozymes in the normal human skin. To determine the location better, epidermis was separated from dermis and immunohistochemistry was carried out. 32 skin specimens were prepared for semiquantitative RT-PCR to examine mRNA expression. 10 of them were separated epidermis from dermis by heat (65 C, 2minutes) for the RT-PCR at each portion. 10 skin specimens were stained with anti-CYP isozyme, 1A1, 2C9, 2E1 and 3A4, antibodies. The results obtained from the RT-PCR were analyzed and compared with those from immunohisto-chemical staining. The condition of heat separation was considered reasonable from the histologic findings and the RT-PCR results. The levels of mRNA expression showed individual variation, but sex and/or age variation was not significant, except for 2C8/19. mRNA was expressed higher in epidermis than in dermis, except CYP2E1. Immuno-histochemistry showed the main location of CYP isozymes was lower portion in epidermis. In dermis, appendages, such as sebaceous glands, hair follicles, sweat ducts and glands were stained more, it may explain the regional difference of therapeutic or adverse effects from topical agents. The results confirmed the skin to be an important organ for drug metabolism and provided a basic data for the study of cutaneous drug metabolism.

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Genetics of Keratinocyte Colony Formation in Mice

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We tested 7 strains of female mice in a quantitative assay for clonogenic keratinocytes having characteristics of stem cells from the cutaneous epithelium. We found three significantly different subsets of colony counts: C57Bl/6>>SENCAR=DBA/2=C3H=BALB/c>CD-1=FVB. Average counts per 1000 viable cells harvested from dorsal skin ranged from 81.5 ± 9.44 for C57Bl/6–29 ± 4.24 for BALB/c to 13.8 ± 2.19 for CD–1 (n = 12 ± SEM). C57Bl/6 and BALB/c, two inbred parental strains were chosen for further analysis. The F1 generation of these two parental strains had an intermediate number of colonies (53.0 ± 6.98; n = 10 ± SEM). Analysis of the parental strains, the two backcrosses and the intercross demonstrates that keratinocyte colony number is quantitative multigenic trait. A genome scan maps the number of keratinocyte colonies to the central region of mouse chromosome 9. The allele on chromosome 9 locus is inherited from the C57Bl/6 parent and appears to influence the high number of keratinocyte colonies. The analysis of keratinocyte colony size in two parental strains, the F1 and the segregating crosses suggests that size of keratinocyte colonies is also a quantitative genetically defined trait. We showed that there are at least two populations of keratinocytes that gave small (less then 2 square mm) and large (more then 2 square mm) colonies, that are associated with different loci. The C57Bl/6 allele of the central region chromosome 9 appears to regulate small colonies (genome wide p-value = 0.01). A locus on the proximal region of chromosome 4 is associated with the large colonies. We suggest that there are at least two subpopulations of cutaneous progenitor cells regulated by two different loci. Investigation of genes regulating stem cell number may play an important role in the development gene therapy or other therapies where it is desirable to manipulate keratinocyte stem cell number.

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Topical Selection of Keratinocytes Transduced with the Multidrug Resistance (MDR) Gene Results in Sustained High-Level Transgene Expression *In Vivo*

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In gene therapy, a clinically relevant therapeutic effect requires long-term expression of the desired gene at a level sufficient to correct the underlying gene defect. One solution would be to select for keratinocytes (KC) expressing both the desired gene and a linked selectable gene, such as the multidrug resistance (MDR) gene, in a bicistronic vector. Utilizing an *in vivo* mouse model to topically select grafts containing MDR-transduced human KC and fibroblasts with colchicine, we determined: (1) Are MDR-transduced KC protected during topical selection? (2) Can topical selection increase the percentage of MDR-transduced cells over time and increase the cellular level of MDR expression? Titration of topical colchicine (100–500 μg per g) on mouse skin and grafted control (MDR-negative) human KC resulted in a dose-dependent increase of KC blocked in mitosis (up to 45%) and eventual ulceration. In constrast, MDR-transduced KC treated with topical colchicine formed an epidermis with normal differentiation and stratification and few mitotic figures (approx. 4%), demonstrating protection from the cytotoxic properties of colchicine. At different time points, colchicine treatment (200 μg per g) of MDR-transduced grafts significantly increased the percentage of human KC expressing MDR compared to vehicle-treated controls; approximately 4-fold by FACS at 9 weeks (49 ± 9% vs. 13 ± 2%) and 10-fold at 15 weeks (50 ± 8% vs. 5 ± 1%). The average level of MDR expression per cell was also increased approximately 2-fold on quantitative FACS analysis. Even when the initiation of topical selection on MDR transduced grafts was delayed, significant enrichment of MDR-expressing KC could be achieved, suggesting selection for MDR-expressing KC stem cells. Interestingly, colchicine selection of grafts containing MDR-transduced human KC with MDR-negative human fibroblasts further increased MDR-expressing KC (80 ± 12%), but had a significant percentage of ulceration (57%), suggesting that fibroblasts are needed for graft support. Thus, topical selection presents a strategy to efficiently increase long-term high level expression of transgenes linked to MDR by utilizing both MDR-transduced KC and fibroblasts and holds promise for future clinical applications.

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Lentiviral Vectors Mediate Highly Efficient and Sustained Gene Transfer in Dystrophic Epidermolysis Bullosa Skin Cells

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Dystrophic epidermolysis bullosa (DEB) is a family of inherited mechano-bullous disorders caused by mutations in the type VII collagen (COL VII) gene and subsequent perturbations in anchoring fibrils. The lack of therapy for DEB provides an impetus to develop gene therapy strategies which can offer highly efficient transfer and stable expression of genes delivered to the skin cells *in vivo*. In this study, we applied a highly efficient lentiviral delivery approach to express both a full-length COL VII α chain and a type VII minicollagen α chain using a self-inactivated third generation lentivirus-based vector, pRRLsinCMV. Transduction of lentiviral vectors containing COL VII transgenes into RDEB keratinocytes and fibroblasts (in which COL VII was absent) resulted in the synthesis and secretion of either a 290-kDa full-length COL VII or a 240-kDa type VII "minicollagen" at levels similar to or higher than normal cells. The expression of the COL VII transgenes was sustained for at least 6 months. Gene-corrected RDEB keratinocytes and fibroblasts demonstrated an enhanced cell-substratum adhesion and an increased proliferative potential. The gene-corrected keratinocytes also showed reversal of the hypermotility phenotype characteristic of RDEB cells. We also carried out a series of gene delivery experiments with cells in culture and compared the transduction efficiencies of the lentivirus-based vector with a retrovirus-based vector, LZRS. By immunoblot analysis of proteins from conditioned media of viral-transduced RDEB cells, the expression of minicollagen using lentiviral vectors was significantly higher than the expression with retroviral vectors. By immunofluorescence staining of the transduced cells with an anti-COL VII antibody, lentiviral vectors achieved a much higher transduction efficiency (80–90%) than retroviral vectors (40–50%). These data indicate that efficient COL VII transgene delivery to keratinocytes and fibroblasts can produce a population of phenotypically corrected RDEB cells. Lentivirus-based vectors are more efficient at transducing human keratinocytes and fibroblasts than retroviral vectors and may be among the most promising approaches to achieve high efficiency and stable gene expression in skin cells.

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Retroviral Gene Therapy Reverses Cutaneous Granuloma Formation in a Murine Model of X-Linked Chronic Granulomatous Disease

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As a result of the inability of their phagocytes to undergo a respiratory burst, patients with the genetic condition chronic granulomatous disease (CGD) develop recurrent infections with catalase-positive bacterial and fungal pathogens, and are predisposed to chronic inflammatory granulomatous lesions in many organs including the skin. CGD can result from mutations in any one of four genes encoding different subunits of the leukocyte NADPH oxidase. Thus, CGD is a candidate disease for gene therapy. Previously, our laboratory has generated a murine model of X-linked CGD by homologous recombinant deletion of the gp91phox component of the NADPH oxidase. Functional studies with these X-CGD mice demonstrated exaggerated inflammatory response which consists of neutrophils which later form granulomas in response to intratracheal, intraperitoneal, or intradermal administration of sterile *Aspergillus fumigatus* (AF) hyphae, in comparison to wild-type mice (*J Exp Med* 185:207, 1997). In our present study, sterile AF hyphae or PBS vehicle were injected into the ears of X-CGD, wild-type control mice, and X-CGD mice who have received therapy with bone marrow cells transduced with a replication-deficient retrovirus encoding the gp91phox gene. Inflammation was assessed by obtaining 5 mm punch biopsies of the injection sites at various times (1–30 days) following injection for weighing and measurement of ear thickness, as well as histologic evaluation. Intradermal injection of AF (but not PBS alone) resulted in a significant ($p < 0.05$, ANOVA) inflammatory response in X-CGD mice by 24 h, with formation of neutrophil-rich granulomas within one week. However, wild-type mice did not exhibit inflammation or granuloma formation over a 30-d period in response to intradermal AF. Both genotypes reacted similarly in a model of allergic contact dermatitis. X-CGD mice which underwent replacement gene therapy were protected against the enhanced reactivity to intradermal AF in comparison to untreated X-CGD mice. AF-induced inflammation in X-CGD mice exhibiting a high level of gene replacement approached that seen in wild-type mice. These studies describe an experimental model system for cutaneous granuloma formation, as well as a clinical functional test for CGD gene therapy.

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Expression of a Single Chain Variant of Human Factor VIII Targeted to Epidermis Corrects Factor VIII Deficiency in Hemophilia A Mice

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Hemophilia A is an X-linked bleeding disorder that results from lack of functional factor VIII protein, making it a good model disease for gene therapy. Epidermis is an attractive tissue for targeting gene therapy because it is highly accessible and gene products expressed there can enter the systemic circulation. Previously, we generated transgenic mice that express human B domain-deleted factor VIII in the suprabasal epidermis under control of the involucrin promoter and bred them into a factor VIII knockout background. These mice had functional circulating factor VIII and displayed phenotypic correction. This demonstrated proof of principle that targeting factor VIII expression to epidermis can correct hemophilia A. We are now exploring strategies for optimizing factor VIII expression in the epidermis to maximize both delivery to and activity in the circulation. One approach is to express factor VIII as a single peptide rather than the normal precursor that is cleaved into heavy and light chains, which form an unstable heterodimer. We have generated a transgene construct that uses the involucrin promoter to express a variant of human B domain-deleted factor VIII that lacks the proteolytic cleavage site at residue 1648. Six transgenic founder mice have been obtained that express this construct as shown by RT-PCR analysis of total RNA from tail tissue. To date, three transgenic lines have been bred into a factor VIII knockout background. Mice from these lines produce functional factor VIII and show average factor VIII activity of 3–39% of normal. We are comparing factor VIII expression, activity and antigen levels of these mice to those of transgenic mice that express standard B domain-deleted factor VIII under control of the involucrin promoter. In this way, we will assess whether epidermal expression of single peptide factor VIII is superior to two-peptide factor VIII for gene therapy.

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Specific Inhibition of Constitutive and Inducible Keratinocyte Gene Expression in Human Skin Following Topical Application of Antisense Oligonucleotides

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Disruption of normal keratinocyte homeostasis commonly occurs in basal cell and squamous cell skin cancers and in inflammatory skin disease. We have previously shown that topical application of a 20mer deoxyphosphorothioate antisense oligonucleotide in a cream formulation results in dose-dependent, sequence-specific, inhibition of tumor necrosis factor-alpha (TNF-α)-induced intercellular adhesion molecule-1 (ICAM-1) protein expression in human skin engrafted on SCID mice. To extend this observation to novel oligonucleotide chemistries, as well as to determine whether topically applied antisense oligonucleotides could be broadly utilized to inhibit gene expression in human skin, we targeted a constitutively expressed gene, Bcl-x, and an inducible cytokine gene, TGFβ1, which are highly expressed in human epidermis. In these studies, we utilized second-generation 2′-O-methoxyethyl (MOE)-modified oligonucleotides that display higher hybridization affinity for target mRNA and greater nuclease resistance, compared to deoxyphosphorothioates. Immunohistochemical analysis showed that topical application of these antisense oligonucleotides to engrafted human skin effectively diminished target protein expression in keratinocytes. Topical application of oligonucleotides in mouse, rat, and pig skin demonstrated rapid absorption of oligonucleotide in epidermis and dermis followed by slow clearance. These studies also showed a favorable safety profile, with only mild and transient erythema observed at higher doses in rats during prolonged exposure regimens. Together, the data indicate that both deoxyphosphorothioate and MOE-modified antisense oligonucleotides potently inhibit gene expression in human skin xenografted onto SCID mice. Pharmacokinetic and toxicological studies suggest that topical administration of antisense oligonucleotides represents an intriguing option for therapy of diseases of the skin. Data addressing the relative potency, duration of action, and systemic exposure of our various proprietary antisense oligonucleotide chemistries following topical administration *in vivo* will also be presented.

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Targeted Gene Correction by Single-Stranded Oligonucleotides in Mammalian Cells

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Previously, we have shown that RNA-DNA oligonucleotides (RDOs) can correct or cause a specific point mutation in episomal and genomic DNA. Frequency of gene correction by RDO was measured using a shuttle vector containing a single point mutation in the LacZ gene. Here, we demonstrate that relatively short single-stranded oligodeoxynucleotides, 25–61 bases homologous to the target sequence except a single mismatch to the targeted base, are capable of correcting a point mutation in the LacZ gene, in nuclear extracts, episome, and chromosome of mammalian cells, with a correction rates of approximately 0.05%, 1%, and 0.1%, respectively. Surprisingly, these short single-stranded oligonucleotides (ODNs) showed similar gene correction frequency to chimeric RNA-DNA oligonucleotide, measured using the same system. The *in vitro* gene correction induced by ODN in nuclear extracts was not dependent on the length nor the polarity of the oligonucleotide. In contrast, the episomal and chromosomal gene corrections were highly dependent on the ODN length and polarity. ODN with a homology of 45 nucleotides showed the highest frequency and ODN with antisense orientation showed a 1000-fold higher frequency than sense orientation, indicating a possible influence of transcription on gene correction. Deoxyoligonucleotides showed higher frequency of gene correction than ribo-oligonucleotides of the identical sequence. These results show that a relatively short ODN can make a sequence-specific change in the target sequence in mammalian cells, at a similar frequency as the chimeric RNA-DNA oligonucleotide.

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Ezrin and Moesin are Involved in Uptake and Trafficking of Naked Plasmid DNA and Oligonucleotides

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As we have already shown that naked plasmid DNA and oligonucleotides are taken up and expressed by keratinocytes, we now show evidence that ezrin and moesin, two members of the cytoskeleton-membrane-linker family are involved in this process. By two dimensional gel electrophoresis and subsequent mass spectrometry (MALDI), ezrin and moesin were found to bind DNA. By immunohistology using monoclonal antibodies, ezrin was found to be expressed in suprabasal layers of the epidermis correlating with the location in epidermis that shows highest uptake of oligonucleotides in immunofluorescence studies. Additionally, monoclonal ezrin and moesin antibodies inhibited one of two oligonucleotide-binding proteins in gel shift analysis, thus further indicating their involvement in the binding of oligonucleotides. The known functional association of ezrin with membrane receptor molecules such as CD44, EGF-receptor and ICAM-2 offers different possibilities of DNA uptake by keratinocytes. Currently, we are investigating the role of such membrane receptor–ezrin associations to elucidate the *trans*-membrane transport of oligonucleotides.

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Transformation-Defective Adenovirus 5 E1A Mutants Exhibit Antioncogenic Properties in Human BLM Melanoma Cells

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Adenoviral E1A proteins exhibit a strong tumor-suppressive activity in human tumor cells. However, E1A is capable of transforming rodent and human cells in cooperation with other oncoproteins, such as activated RAS. Thus, the therapeutic use of wild-type E1A harbors the principal risk of enhancing tumor malignancy. This prompted us to construct E1A13S cDNA-derived mutants that were unable to transform baby mouse kidney cells in cooperation with E1B and to test their tumor-suppressive activity in BLM human melanoma cells. Anchorage-independent growth in soft agar was reduced for those cell lines expressing the E1AdelCR2 mutant, which lacks the entire conserved region 2 (CR2) sequences, or for cells expressing the E1AcR3Ex2 mutant, which contains CR3 plus exon 2 sequences. In contrast, cell lines expressing the entire E1A wild-type (E1Awt) or only the exon 2 sequences (E1AEx2) grew like the parental BLM cells. Moreover, inoculation of nude mice with BLM cells or cells expressing E1AEx2 revealed large tumors after 2 weeks. In contrast, tumors derived from E1AdelCR2- or E1ACR3Ex2-expressing cells exhibited a substantial delay in tumor growth accompanied by loss of E1A expression in the outgrowing tumors. Cell lines expressing E1Awt showed an intermediate phenotype. Thus, expression of CR3 plus exon 2 sequences is sufficient to enhance both the antioncogenic properties and the therapeutic safety of E1A in the experimental model tested.

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The Gene Peel: Gene Gun Delivery of the Diphtheria Toxin Fragment A (DTA) Gene to Mouse Skin Produces Epidermal Blister

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Diphtheria toxin fragment A (DTA) is a well-characterized toxin that inhibits protein synthesis by ADP-ribosylation of elongation factor-2, resulting in cell death. Selective killing of various cell types can be accomplished through transcriptionally controlled expression of this gene using expression vectors with different promoters. We have previously demonstrated that the human involucrin promoter can produce high levels of transgene expression *in vivo*, as assayed by β-galactosidase reporter gene. In this study, an expression vector was constructed containing the human involucrin promoter to direct the expression of DTA. The plasmid DNA was delivered to adult BALB-C mouse skin by gene gun. Serial histological examination over 48 h revealed the evolution of an epidermal blister associated with a dense neutrophilic and lymphocytic inflammatory cell infiltrate. The inflammation and necrosis was primarily in the epidermis, with minimal involvement of the dermis. Epidermal blisters induced by gene delivery can be thought of as a "gene peel". The unique characteristic of the gene peel is the molecular control of gene expression conferred by the promoter used in this expression vector. The gene peel may provide an accurate modality for the ablation of specific layers of the epidermis and dermis.

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Transduction of Graftskin with a Modified Herpes Vector

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Graftskin is a bilayered organotypic skin culture consisting of differentiating neonatal derived keratinocytes layered over neonatal fibroblasts that are embedded in a bovine collagen matrix. In previous studies, we have shown that graftskin can be transduced by replication deficient retroviruses upon wounding. This work illustrated the potential of utilizing bioengineered skin grafts for gene therapy purposes and showed that these grafts could be genetically modified after production. In the present study we used a modified replication deficient herpes virus, THZ.4, as another potential vector for gene transfer to bioengineered skin. Infection of Graftskin by THZ.4 could be shown when the virus was placed on wounded and nonwounded grafts. Unlike retrovirus, expression of the herpes virus transgene did not require wounding. Infection of grafts could be demonstrated histologically with viral titers as low as low as 1×10^4 p.f.u. per ml. Gross expression of the transgene however, required higher viral titers. Cytotoxicity was demonstrated in keratinocytes both at high and low titers of viral infection. At higher titers there was cytotoxicity of both keratinocytes and fibroblasts in the graft. These findings demonstrate limitations of utilizing herpes virus as a gene therapy vector. Less toxic vectors will need to be developed if herpes virus is be utilized in bioengineered skin products. The findings also further illustrate that Graftskin can be easily transduced by viral vectors and can demonstrate cell toxicity due to viral infection in a manner similar to normal skin.

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The Microenvironment Affects Transgene Expression by Human Fibroblasts *In Vivo*

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Using genetically modified fibroblasts (GMFb) we have demonstrated that at least two factors influence transgene expression (TGE) *in vivo*: the inherent life span – immortalized (3T3) cells do not lose TGE; and the microenvironment? maturing GMFb in a dermal matrix *in vitro* enhances TGE *in vivo*. To further assess the microenvironment we postulated that a dermal matrix substrate (an empty nylon matrix? ENM) matured *in vivo* would enhance TGE by GMFb *in vivo*. *In vivo* generated matrixes were generated by transplanting ENM to athymic mice. Later, 4 week, these IV/ENM were harvested and the resident murine cells were killed via 3 rapid freeze-thaw cycles. These IV/ENM were seeded with GMFb and held in tissue culture favorable to human fibroblasts for 4 week before transplantation to athymic mice. Controls were GMFb seeded into an ENM and matured *in vitro* for 4 week. Two sets of experiments have been carried out. IV/ENM and the ENM were loaded with human fibroblasts that were transduced with either lacZ or human transferrin (hTf) and then subjected to cloning by limiting dilution (hFb.T.lacZ.c or hFb.T.hTf.c). These were transplanted to athymic mice and analyzed for TGE. Expression of lacZ by the GMFb after 4 & 8 week *in vivo* was >> in the IV/ENM than in the ENM. Blood levels of hTf were measured in mice implanted with an IV/ENM or an ENM containing 1 million hFb.T.hTf.c; the respective hTf levels were 283 vs. 41 ng per ml @ 4 week, 56 vs. 7.8 ng per ml @ 8 week and 43 vs. 6.7 ng per ml @ 16 week. By 24 weeks blood levels in mice with either construct were at baseline,? 12 ng per ml. At the time of implantation the amount of hTf in the media was 2803 ng per ml for the IV/ENM and 2514 ng per ml for the ENM, not a significant difference. Transgene expression is more robust *in vivo*, from 2.3 to 7.7 fold, by GMFb that are in a transport system that carries an *in vivo* generated microenvironment. However, despite this positive effect there is, with time, a seemingly inexorable loss of TGE by normal human GMFb *in vivo*.

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Exogenous Gene Expression in Skin-Equivalent Keratinocytes using a Cre/loxP Adenovirus System

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The adenovirus vector system is one of the most effective techniques for transducing exogenous genes in normal human keratinocytes, which are suitable for *ex vivo* gene therapy. For safe gene therapy, it is very important to regulate the expression of transduced genes. To regulate transduced gene expression in normal human keratinocytes, we used the Cre/loxP adenovirus vector system. A stuffer sequence, consisting of a neomycin-resistant gene and polyA flanked with loxP, was interposed between the CAG promoter and the coding region for enhanced green fluorescent protein (EGFP). Co-infection with Ad-expressing nuclear-localizing-signal-tagged Cre recombinase removed the stuffer sequence and turned on the expression of EGFP. EGFP expression was detected 6 h after infection and reached a plateau at 24–36 h in monolayer normal human keratinocytes. Initially, to express EGFP in skin-equivalent keratinocytes, we infected keratinocytes with Ad before seeding them on collagen gels. However, expression was limited to the cornified cell layer when the skin equivalent was completely formed. Therefore, we changed the method. Keratinocytes were infected with Ad after the skin equivalent was completely formed. Using this new approach, strong EGFP expression was detected in the basal and suprabasal layers. The Cre/loxP Ad system is a powerful way to express exogenous genes in monolayer and skin-equivalent keratinocytes. This excellent method will enable us to investigate the function of the object genes, to develop disease models using human keratinocytes, and to evaluate gene therapy methods using skin equivalent.

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Tetracycline Inducible Gene Expression for Studies of Progession in Melanoma Cell Lines

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The availability of human melanoma cell lines representing different stages of progression provides the opportunity for transfection studies to assess how various transgenes may contribute to increased or decreased malignancy. For example, we previously showed that expression of activated ras in the RGP melanoma cell line, WM35, confers a VGP-like phenotype (Fujita *et al*, *Melanoma Res* 9:279, 1999). Similarly, others have shown that expression of RhoC in the more advanced melanoma line, A375, markedly increased the metastatic capability of these cells in nude mice (Clark *et al*, *Nature* 406:532, 2000). In these studies, the transgenes of interest were overexpressed from strong, constitutive viral promoters. The use of inducible transcription systems allows detection of more subtle phenotypic effects. For this purpose, we have introduced the positive TET transactivator, rtTA, into both WM35 and A375 cells (Pacheco *et al*, *Gene* 229:125, 1999). We encountered considerable difficulty in isolating stable transfectants of the RGP, WM35 cells by various methods attempted. However, using a retroviral vector, we have now successfully obtained a population of WM35rtTA that shows 50 fold induction of a TET controlled reporter in response to doxycycline. Similarly, our clones of A375rtTA display 30–200 fold reporter induction. We are in the process of transducing various TET controlled transgenes into both of these cell types. The specific goals are to determine effects of regulated expression of Ras and its downstream effectors on the RGP to VGP transition in WM35, and of RhoC and other candidates for promoting metastasis in A375 cells. To facilitate detection of metastasis we have further modified the A375rtTA cells to express uniformly the green fluorescent protein, EGFP, using retroviral transduction and FACS.

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Stability of Transgene Expression by Genetically Modified Immortalized Fibroblasts in an Encapsulated Eevice in the Subcutaneous Space of Athymic Mice

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Previously we have reported on the development of an easily retrievable encapsulated cell delivery device for studying mechanisms of loss of transgene expression (TGE) following the transplantation of genetically modified fibroblasts (GMFb) to an *in vivo* setting. We have demonstrated that TGE by normal human GMFb (lacZ) in this device is robust for 4 week. Thereafter, however, there is loss of TGE without a loss in viability. Previously we reported that GMFb in an open system lose TGE over time with senescent GMFb losing TGE more rapidly than younger GMFb. Further, we have shown that 3T3 cells in this open system do not lose TGE *in vivo* but grow into tumors. For comparison we analyzed TGE by spontaneously immortalized 3T3 cells in the encapsulated device *in vivo*. 3T3 cells were transduced with a retroviral vector encoding human transferrin (hTf) and cloned for TGE by limiting dilution (3T3.T.hTf.c). One million of the 3T3.T.hTf.c cells were loaded into a device and 4 were implanted in the subcutaneous space of athymic mice. Blood was collected at regular intervals and analyzed for hTf. Levels of hTf (ng per ml) increased throughout the time of implantation, mean levels @ 2, 6 and 10 week were 92, 130 and 610, respectively. Devices were harvested and analyzed for viability and TGE. There was no loss of TGE, and all cells were viable on recovery. We conclude that in contrast to nonimmortal GMFb immortalized GMFb do not lose TGE *in vivo*. These observations support the concept of a hierarchical control TGE by fibroblasts carrying stably integrated transgenes *in vivo*. We have reported that one of these hierarchical element is the microenvironment; GMFb in an *in vivo* generated microenvironment have TGE that is than one generated *in vitro*. It appears that another of the hierarchical elements is the inherent life span of GMFb.

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Use of a Novel Keratinocyte Cell Line, NIKS, for Investigation of Skin-Based Gene Therapies

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Human skin is a readily accessible tissue for gene therapy. Others have reported that human keratinocytes can be engineered to secrete exogenous bioactive molecules. These genetically engineered keratinocytes have been successfully grafted onto host animals, supporting the contention that skin is an attractive target tissue for gene therapy. However, long-term, stable expression of exogenous genes has not been achieved in part because of the keratinocyte terminal differentiation program. Differentiating keratinocytes transfected with a transgene are eliminated from the tissue over time. In this report we use a novel human keratinocyte cell line, NIKS cells, which possesses the growth and differentiation characteristics of normal human keratinocytes. We have optimized stable transfection of the NIKS cell line using Green Fluorescent Protein (GFP)-expressing plasmids to identify stably transfected cells. Using Flow-Activated Cell Sorting (FACS), transfected NIKS keratinocytes were isolated in the absence of toxic selective agents which promote cell death of this cell type. To investigate the ability of GFP-expressing keratinocytes to differentiate and stratify, we constructed organotypic cultures that closely resemble the three-dimensional microenvironment of human skin. Organotypic cultures of GFP-expressing NIKS cells were transplanted onto immunocompromised animals for further analysis of gene expression. We hypothesize that the normal growth and differentiation characteristics of the NIKS cells, coupled with immortality, will allow for stable long-term expression of GFP and other transgenes. This report demonstrates that the human NIKS cell line represents an important new cellular reagent for the development of gene therapy strategies in stratified squamous epithelia such as skin.

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A Long-Term Prospective Survey of Atopic Dermatitis and Hand Eczema in a University Clinic

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To provide a systematic approach to the diagnosis and evaluation of patients with inflammatory skin disease, we designed a standardized evaluation form to prospectively record historical and physical characteristics of patients with possible atopic dermatitis (AD). During a 20-year period, we obtained a compilation of features during the initial visit of 950 patients to the Oregon Health Sciences University Dermatology Clinic. Stratified by age, 67.7% were 12-year-old, 17.3% were 3–12-year-old, and 15.0% were 0–2-year-old at the time of evaluation. By history, 57.8% of the patients had presenting signs and symptoms before age one. The mean age of onset was approximately 4.3 years. Associated allergic respiratory disease was present in the majority of patients regardless of age. Seventy-three percent of patients had a history of asthma and/or allergic rhinitis with the latter found more commonly in all age groups. Among the 626 patients diagnosed with AD who were two years old, 498 (79.6%) had a history of flexural eczema, compared to 62.2% in children 0–2-year-old. Regarding nonspecific physical features, signs of ichthyosis were present in more than 50% and scalp involvement in 37.8%. Active hand eczema was documented at the time of examination in 61% of patients. Hand eczema tended to begin within one year of the initial onset of AD. Hand eczema tended to involve primarily dorsal surfaces in all age groups. However, web space and palmar involvement were more frequent in older age groups compared to younger age groups. Vesicular lesions of the palms and sides of fingers were not uncommon. This study establishes the frequency of physical and historical characteristics for diagnosis in a large cohort of patients with AD and indicates a reservoir of active or potential hand eczema in this very common disease.

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Third-Party Payer Cost of Atopic Dermatitis and Eczema in the United States

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Atopic dermatitis/eczema (AD/E) is a common disease. Few studies have attempted to quantify the cost of disease to third-party payers. Our objective was to identify the annual cost to third-party payers of medical services and prescription medications for the treatment of AD/E. A retrospective study was conducted using claims data from 1997 and 1998 from: (1) a private insurer, and (2) a state Medicaid program. Beneficiaries were considered to have AD/E if they had at least one claim in 1997 with a primary or secondary listing of one of three diagnosis codes: (1) 691.8, other atopic dermatitis and related conditions; (2) 692.9, contact dermatitis and other eczema unspecified cause; or (3) 373.3, noninfectious dermatoses of eyelid. Patients who did not meet these criteria were evaluated as part of a "control" group in each payer for comparisons of expenditures with the AD/E group. Outcome measures were disease prevalence and total health care costs incurred by each third-party payer. Disease prevalence was 2.4% (private insurer) to 2.6% (Medicaid) of all eligible beneficiaries and 3.5% to 4.1% of patients submitting at least one health care claim during the study period. The third-party payer cost of illness for AD/E ranged from $0.9 to $3.8 billion when projected across the total number of people under age 65 insured by private insurance and Medicaid in the US. Nearly one-third of all health care costs for patients with AD/E may be attributed to AD/E and comorbid conditions. In conclusion, the annual costs of AD/E are similar to those of diseases such as emphysema, psoriasis, and epilepsy. AD/E patients incur significant costs associated with comorbid conditions.

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SDZ ASM 981 Cream 1% is Efficacious and Safe in Infants Aged 3–23 Months with Atopic Dermatitis

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Introduction: SDZ ASM 981, a selective inhibitor of inflammatory cytokine release has been shown to be effective in the treatment of adult subjects and children 2 years or older with atopic dermatitis (systemic exposure in adults and children is consistently low, even when applied over large body surface areas). A multicenter vehicle-controlled study in infants from 3-23 months with atopic dermatitis was conducted to evaluate the efficacy and safety of SDZ ASM 981 Cream 1%. Methods: Subjects with mild-to-moderate atopic dermatitis were randomized to BID treatment for up to 6 weeks with SDZ ASM 981 Cream 1% (ASM) or the corresponding vehicle (VEH) (2:1 randomization). Efficacy was assessed by an Investigators' Global Assessment (IGA), the Eczema Area and Severity Index (EASI), and assessment of pruritus. Safety was assessed throughout treatment via reporting of adverse events and monitoring of laboratory tests and vital signs. 186 subjects were enrolled into the study, an interim analysis of the first 83 patients is herein reported. For this interim analysis the two-sided significance level for testing treatment differences was set at 0.005 (O'Brien Fleming). Results: No demographic differences were noted between treatment groups. Fifty-six (95%) of the ASM subjects completed the study while only 12 (50%) of the VEH subjects completed the study. 62.7% of ASM subjects achieved treatment successes (IGA = 0 or 1, i.e. clear or almost clear) compared to 16.7% VEH subjects ($p<0.001$). Treatment effect was rapid, with statistical significance being reached as early as Day 15 (47.5% ASM; 16.7% VEH; $p=0.004$). Mean reduction in EASI at the end of study was significantly greater for ASM-treated subjects than for VEH-treated subjects. Significantly more subjects on ASM had little or no pruritus (score of 0 or 1) than on VEH, a difference noted early in treatment. Treatment effects were consistent across all measures and were maintained or improved over treatment course. ASM was well tolerated: no patients discontinued for any adverse event. Only 2 subjects in each treatment group had any application site adverse events. No systemic or serious adverse events were noted. Conclusions: This study provides evidence of SDZ ASM 981 cream 1% being safe and significantly more effective than its corresponding vehicle in the treatment of atopic dermatitis in infants.

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Improvement in Recalcitrant, Childhood Atopic Dermatitis with a Ceramide–Dominant Barrier Repair Moisturizer

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Currently, it is fashionable to consider atopic dermatitis (AD), like many other inflammatory dermatoses, as immunologic in etiopathogenesis (''inside-outside'' hypothesis). Indeed, drugs such as topical glucocorticoids and immunosuppressives are mainstays of therapy. Yet, the risk of side-effects and toxicity from these agents is not insignificant, particularly in children. Alternatively, the pathogenesis of AD has been linked to the permeability barrier abnormality, which could drive disease activity (''outside-inside'' hypothesis). Although moisturizers are mainstays of therapy, current products do not correct the underlying ceramide (Cer) deficiency in AD. Hence, we assessed here the efficacy of a new over-the-counter, Cer-dominant, physiologic lipid-based moisturizer (TriCeram, Osmotics Corp.) in 21 children, aged 2–16, with stubborn-to-recalcitrant AD. All were receiving optimal therapy (tacrolimus or topical steroids, antihistamines, standard moisturizers). To avoid seasonal bias, all were enrolled during Oct–Nov, and all continued their ongoing therapy, only substituting TriCeram for their prior moisturizer. SCORAD scores improved significantly in 19 of 21 patients by 3 weeks, with further improvement by 6 weeks in the group as a whole (50%). Transepidermal water loss levels, which were elevated over involved and uninvolved areas at entry, decreased significantly in parallel with SCORAD scores, while SC integrity (cohesion) improved significantly. Finally, ultrastructure of TriCeram-treated SC revealed extracellular lamellar membranes, which were largely absent at baseline. These studies suggest that a Cer–dominant, barrier repair moisturizer is a useful adjunct in the therapy of stubborn AD. Finally, these studies support the ''outside-inside'' hypothesis of disease pathogenesis in AD, suggesting that barrier repair moisturizers, based upon optimal ratios of the three key SC lipids, represent an alternate, safe approach to the therapy and prevention of inflammatory dermatoses.

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The Role of Corneotherapy in the treatment of Skin Barrier Damage Due Allergic Contact Dematitis

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Background: Nickel-induced allergic contact dermatitis (ACD) using a contact allergy patch (CAP) test has been shown to be associated with skin barrier alterations. In human volunteers, CAP tests combined with skin bioengineering methods including transepidermal water loss (TEWL) and stratum corneum (SC) hydration measurements, and visual scoring have been used to quantify the effect of topical agents on ACD. Objective: The aim of this study now is to compare in ACD the efficacy of combination therapy, using a potent topical corticosteroid (fluticasone proprionate 0.05%) and a corneotherapy agent (barrier cream), with that of a single therapy of the corticosteroid emollient alone. Methods: 14 Nickel patch-test-positive female volunteers with a mean age of 31.5 (range 24-36) were included in the trial. On Day 1, six Extra Large Finn Chambers were symmetrically applied on forearm skin for 48 h. Four of these Chambers contained 5 % nickel sulfate in petrolatum in order to create a standardised dermatitis. The two remaining chambers contained physiologic saline and served as a control. Either the topical corticosteroid cream or the barrier cream was applied twice daily and matched with the combination of topical corticosteroid (once daily at 0 hour) and barrier cream (once daily at 12 hours). This application method was undertaken on three of the four nickel-ACD skin areas. The fourth nickel-ACD test spot served as a non-treated control. Clinical scoring, TEWL, and skin hydration measurements were done at day 1, before applying the patch tests, and after the induction of ACD, namely on days 4 to 8. Results: Combination therapy proved to provide superior results than those obtained using the corticosteroid alone twice daily, the barrier cream alone twice daily or no treatment at all (the later being the physiological recovery of the skin barrier function after induction of ACD). SC hydration showed better results under both combination therapy and barrier cream (twice daily) application, than those found when the topical corticosteroid was applied twice or the physiological recovery of the non-treated control was considered. Conclusion: This study demonstrates that combining classical corticosteroid anti-inflammatory therapeutics with corneotherapy agents can optimise the healing capacity of the human skin barrier function in ACD. Therefore, combination therapy can lead to new methodologies in the topical therapy of inflammatory dermatosis (contact allergy, atopy, psoriasis) characterised by an abnormal barrier function.

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Interleukin-1β Converting Enzyme (Caspase-1) Inhibition with VX-765 Reduces Inflammation and Cytokine Levels in Murine Oxazolone-Induced Dermatitis

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There is evidence for key roles of the cytokines interleukin-1β (IL-1β) and IL-18 as immune surveillance activators and etiological factors in dermatoses involved in the recruitment of macrophages, lymphocytes, neutrophils and eosinophils. Thus, a potential target for therapy in dermatoses is IL-1β converting enzyme (ICE, Caspase-1), the proteolytic enzyme that processes and activates IL-1β and IL-18 from inactive precursors. We tested this hypothesis using an orally bioavailable ICE inhibitor, VX-765. Delayed-type and immediate-type skin hypersensitivity to oxazolone was induced in mice by sensitization on the abdomen on day 0 and challenge on one ear at day 3, as a model of allergic dermatitis. VX-765 (10–100 mg per kg *po bid*) administered before or after oxazolone challenge dose-dependently reduced oxazolone-challenged ear edema by up to 75% (24–94 h postchallenge). Significant reduction of thickening and cellular infiltration was observed in both the dermis and epidermis. As expected, the levels of mature IL-1β and mature IL-18 in ear homogenates were reduced by up to 90% and 75%, respectively. Furthermore, tissue levels of major downstream inflammatory and allergic cytokines and chemokines were reduced (% inhibition in a representative experiment): IFNγ (81%), IL-4 (75%), MCP-1 (74%), MIP-1α (94%), MIP-2 (99%), as well as myeloperoxidase (80%) and nitric oxide (72%). These data suggest that both the Th1 (delayed-type) and the Th2 (immediate-type) skin hypersensitivity can be blocked by targeting ICE, thereby preventing the initiation and propagation of the neutrophil-mediated inflammatory cascade. The effects of ICE inhibition on the eosinophil-mediated allergic cascade remains to be investigated. ICE inhibition appears to be a promising strategy for the treatment of inflammatory and autoimmune skin diseases.

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Are Corticosteroids of Clinical Value in Lipid-Soluble Induced Irritation in Man?

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Introduction: Topical corticoids are used to treat irritant contact dermatitis (ICD) in man. However, their clinical efficacy remains *sub judice*. Objective: We studied the effects of low and medium potency corticosteroids on lipid soluble-induced ICD in man. Methods: ICD was induced by 24-h patch application of nonanoic acid (NAA) onto volunteers' forearms. Betamethasone-17-valerate, hydrocortisone, and petrolatum control were applied twice daily. Visual grading, transepidermal water loss (TEWL), laser doppler flowmetry (LDF) and chromametry quantified responses on days 1-5 and 8. Results: On day 8, with 90% NAA, a slight, yet statistically significant improvement in TEWL was observed with betamethasone when compared to untreated control. Betamethasone also significantly decreased chromametric values on day 8 with 90% NAA when compared to hydrocortisone. Petrolatum reduced LDF when compared to untreated control at 60% NAA on day 3. Conclusion: The results suggest a slight improvement with betamethasone and petrolatum though their benefit in typical clinical use remains unclear.

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SDZ ASM 981 is Highly Effective in Animal Models of Skin Inflammation, but has Only Low Activity in Models Indicating Immunosuppressive Potential, in Contrast to Cyclosporin A and FK 506

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SDZ ASM 981 is a selective inhibitor of inflammatory cytokine release, specifically designed for the treatment of inflammatory skin diseases. The purpose of this study was to compare SDZ ASM 981 with the immunosuppressants cyclosporin A (CyA) and FK 506 after systemic application in models of T cell mediated skin inflammation (allergic contact dermatitis, ACD) and models of localized graft vs. host (GvH) reaction and allogeneic kidney transplantation in order to assess the immunosuppressive potential. In murine ACD, SDZ ASM 981 was as effective as FK 506 (ED50: 48 mg per kg, p.o.) and superior to CyA (ED50: 90 mg per kg, p.o.). In rat ACD, the lowest oral doses with significant effects were 12.5 mg per kg for SDZ ASM 981 and 50 mg per kg for CyA. FK 506 had no significant effect even at a dose of 25 mg per kg, p.o. The GvH reaction was inhibited by FK 506, CyA and SDZ ASM 981 in a dose-dependent fashion after sc. application. The ED50s were 0.3 mg per kg for FK 506, 2.5 mg per kg for CyA and 20 mg per kg for SDZ ASM 981. In the transplantation model, the lowest oral dose at which animals survived 100 days or longer was 15 mg per kg for SDZ ASM 981. In comparison, 5 mg per kg CyA and 1 mg per kg FK 506 were required to achieve the same long-term survival after oral dosing. In summary, SDZ ASM 981 turned out to be equally effective or superior to FK 506 and superior to CyA in murine and rat ACD. CyA and FK 506 were superior to SDZ ASM 981 in suppressing GvH reactions by a factor of 8 and 66, respectively, and in kidney transplant rejection by a factor of 3 and 15, respectively. These data indicate, that SDZ ASM 981, unlike CyA and FK 506, has a skin selective pharmacological profile.

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Comparative Effect of Different Generic Brands of Fluocinolone Acetonide Cream in an *In Vivo* Model of Provoked Skin Irritation with Sodium Lauryl Sulphate

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All over the world, the generic drug market is in the process of expansion. Regarading topical corticosteroids, there are several preparations but their clinical efficacy has been poorly assessed in relation with the original brand name in an experimental model of skin irritation *in vivo*. Using fluocinolone acetonide cream, we evaluated the anti-inflammatory effect of four different brand as follow: the original brand, generic A, generic B, and generic C. A double blind, vehicle-control essay was conducted after producing irritation on five sites of the volar aspect of forearm in 20healthy volunteers. For the purpose of creating skin irritation, 10 µL of 10% aqueous sodium lauryl sulfate were applied in a Finn Chamber during a 24-h period. After that, aliquots of the four brands of fluocinolona cream were applied and occluded during a four-day period. Readings were made according the scale of Frosch and Kligman at the second and fourth day. The results collected on the second day showed that one of the generic preparations (C) displayed a score not statistical different than the control (4.4 vs. 5.25 $p = 0.05$) and this tendency was also kept through the forth day (4.45 vs. 5.62 $p = 0.05$). The rest of those preparations were as effective as the original one in controlling inflammation. The analysis showed that although the cost of the generic drug (C) was 80% less than the original medication, the clinical response was not as good as the others preparations. After these results, the following question may arise: Is it necessary to perform clinical studies with the generics in a similar way in which it is being done with the original preparations in order to confirm their effectivity before marketing? This deserves some consideration, as newer and more potent generic drugs will be available in the future.

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Increased bcl-2 Gene Expression in Severe Atopic Dermatitis *In Vivo*

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The purpose of our study was to investigate the baseline expression of the apoptosis inhibitor gene bcl-2 within the dermal inflammatory infiltrate referring to skin biopsies of patients suffering from severe atopic dermatitis. Punch skin biopsies of involved skin were obtained from 15 patients with acute exacerbation of severe atopic dermatitis. All samples were tested immunohistochemically for features of bcl-2 expression. In order to ensure an over-expression, sections were compared with healthy controls. With this immunohistochemical study, we succeeded in the detection and the quantitative assessment of the expression of bcl-2 in atopic dermatitis *in vivo* in contrast to healthy controls. Atopic dermatitis is a chronic inflammatory skin disease characterized by severe pruritus, typical eczematous morphology and a chronic relapsing course. Histologically, it is recognized by an increased number of T lymphocytes, predominantly $CD4^+$ T-helper cells of the Th_2-subtype, connected with the expression of a specific cytokine pattern. Within the last years, the discovery of various gene families being responsible for the regulation of pro- and antiapoptotic mechanisms led to the development of new staining methods for the detection of lymphocytes undergoing the programmed cell death, as well as for cells, which develop strategies to circumvent the physiological pathways. Among these findings the bcl-2 gene expression is of pronounced interest representing a potential antiapoptotic status in sites of cutaneous inflammation. The data obtained by our immunohistological examination demonstrated a high baseline percentage of bcl-2^+ cells within the dermal inflammatory infiltrate in atopic dermatitis. Referring to the special characteristics of the apoptosis inhibitor gene bcl-2, we conclude that this modulation of the low baseline expression in healthy skin may be responsible for the persistence of inflammation in atopic dermatitis.

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Tacrolimus 0.1% Ointment in the Treatment of Eyelid Dermatitis

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Tacrolimus 0.1% ointment (protopic) is a nonsteroidal treatment for atopic dermatitis, that has been used to safely and effectively treat the face. Localization of atopic dermatitis to the eyelids is common. Until now, the main treatment for eyelid dermatitis has been the topical application of corticosteroids. Use of corticosteroids around the eyes has been associated with the development of glaucoma and cataracts as well as local cutaneous side-effects, such as atrophy and formation of telangiectasia. We therefore conducted an open-label trial of tacrolimus ointment in 20 patients with eyelid dermatitis. Patients were treated with tacrolimus ointment twice daily for up to eight weeks. The severity of eyelid dermatitis was assessed at baseline, at regular intervals throughout the treatment period, and at two weeks post-treatment. Four eye exams, including intraocular pressure measurements, were performed before, during, and after treatment. The results of our study demonstrate the safety and efficacy of tacrolimus ointment in the treatment of eyelid dermatitis.

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Intermittent Dosing with Topical Fluticasone Propionate Delays the Time to Relapse in Adults and Children with Chronic Atopic Dermatitis – Two Randomised Controlled Studies

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A recent study (van der Meer *et al*, *BJD*, 1999) suggested that intermittent topical fluticasone propionate (FP) reduces frequency of relapse of chronic atopic dermatitis (AD); two large randomised, controlled studies have been conducted to investigate further the effectiveness of this strategy. A total of 643 patients (231 children, 412 adults) with a history of recurrent AD were treated during an exacerbation with daily FP for 2–4 weeks. Stabilised patients entered a double-blind Maintenance Phase and received regular daily emollients plus intermittent FP or intermittent placebo for up to 20 weeks. Median time to relapse and relative risk of relapse were calculated. In the first study FLTB4012 (a European study, adults only) median time to relapse in both intermittent placebo treated groups was 6.1 weeks. Median time in the intermittent FP treated groups could not be estimated, but was in excess of 16 weeks (the majority of patients remained relapse free at this time-point). Patients were 5.76 times (95%CI 3.08,10.77, $p < 0.001$) and 1.94 times (95%CI 1.17,3.24, $p < 0.01$) more likely to relapse on placebo cream and ointment, respectively, than on intermittent FP. In study FPC40002 (a US study, children and adults, cream only) median time to relapse on intermittent placebo was 5.1 weeks for children and 4.1 weeks for adults. Median time to relapse on intermittent FP cream could not be estimated, but exceeded 20 weeks. Patients were 8.1 (95%CI 4.3,15.2, $p < 0.0001$) times and 7.0 (95%CI 3.0,16.7, $p < 0.0001$) times more likely to relapse on placebo than intermittent FP (children and adults, respectively). There was no evidence of increased risk of skin atrophy with FP. These studies confirm that once an acute episode has been treated effectively, intermittent dosing with FP in addition to regular use of emollients significantly prolongs the relapse free period, delaying the time to relapse in children and adults with moderate to severe chronic AD.

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SDZ ASM 981 Cream 1%: A New Approach to Long-term Management of Atopic Dermatitis

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SDZ ASM 981 Cream 1%, a selective inhibitor of inflammatory cytokine release, was found to be safe and effective in the treatment of atopic dermatitis (AD). To assess long-term safety and efficacy in children (2-to-< 18 years of age) a multicenter, parallel group, double-blind, controlled, 1-year study was performed. 713 AD patients were randomized to either SDZ ASM 981 Cream 1% or corresponding vehicle (2:1), to be applied bid according to need. Emollients were allowed, as were medium-high potency topical corticosteroids (CS) for flares not controlled by study medication (SM). CS were applied according to label. Following treatment with CS, treatment with SM was resumed. Consequently, the control treatment (i.e. emollient, vehicle cream, and CS) was equivalent to current standard care. The vehicle treatment was included in order to maintain the study blind. A flare was defined as an Investigator's Global Assessment (IGA) of 4 (severe) or 5 (very severe). Primary efficacy analysis was conducted on the incidence of flares observed in 6 months, adjusting for discontinuations. SDZ ASM 981 Cream 1% reduced the incidence of flares compared to the control group ($p < 0.001$). 61% of patients in the SDZ ASM 981 Cream 1% group completed 6 months without a flare, compared to 35% in the control group. CS use was less, and time to first use was greater in the SDZ ASM 981 Cream 1% group compared to the control group. Treatment with SDZ ASM 981 Cream 1% was significantly more effective than the control in all secondary efficacy assessments. The incidence of adverse events (AEs) in the SDZ ASM 981 Cream 1% group was comparable to that observed in the control group, indicating a good safety profile with a low overall incidence of AEs. Hence, SDZ ASM 981 Cream 1% has significant therapeutic advantages over the current treatment paradigm (i.e. emollients & topical CS) in the acute and long-term management of pediatric AD.

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Modulation of Cathepsin G Gene Expression in Severe Atopic Dermatitis Following Medium-Dose UVA1 Phototherapy

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During the last decade, medium-dose UVA1 phototherapy (50 J per cm^2) has achieved great value within the treatment of severe atopic dermatitis (AD). The purpose of our study was to investigate to what extent UVA1 irradiation is able to modulate the status of protease activity by the use of a monoclonal antibody labeling cathepsin G. In order to further elucidate the mechanismus by which medium-dose UVA1 irradiation leads to an improvement of the skin status in patients with AD, biopsy specimens from 15 patients before and after treatment were analysed immunohistochemically for features of proteolytic activation. As compared with lesional skin of patients with AD before UVA1 irradiation, the number of cells being positive for cathepsin G within the dermal infiltrate has significantly decreased after treatment. The decrease of cathepsin G^+ cells was closely linked to a substantial clinical improvement of skin condition. In summary, our findings demonstrated that medium-dose UVA1 irradiation leads to a remarkable modulation of the expression of cathepsin G in the dermal inflammatory infiltrate in patients with severe atopic dermatitis. As the serine protease cathepsin G is known to attack laminin, proteoglycans, insoluble fibronectin and collagen I, to induce postinflammatory events, to degrade the basement membrane, to increase the permeability of endothelial barriers and to destroy the tissue inhibitor of metalloproteinases, its down-regulation by UVA1 phototherapy may contribute to the reduction of skin inflammation combined with the remarkable improvement of the skin status.

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Xerotic Dry Skin of the Elderly. A Summer vs. Winter Comparison Based on Non-Invasive Measurements of Barrier Function

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Dry xerotic skin is an inevitable feature of the aging process presenting as rough, dry scaly skin, often accompanied by intense pruritus, especially on the legs. We sought to explain why the condition ameliorates in summer time. We used noninvasive bioengineering methods to compare leg skin parameters in 8 healthy women, ages 63–78, suffering from winter xerosis, with the following results: (1) Transepidermal water loss (Evaporimetry). Diffusional water loss was significantly greater in the winter. (2) Estimation of scaling by serial D-Squames. In winter, more scales were removed by each of 3 adhesive coated discs, reflecting a looser horny layer. (3) A 24-h occlusive application of 0.75% SLS caused a greater inflammatory reaction in wintertime, accompanied by a significant increase in Laser Doppler blood flow, along with increased erythema by Minolta colorimetry. (4) Exposure to 20 : 80 chloroform:methanol for a few minutes resulted in a greatly increased burning sensation in winter time. (5) Application of 90, 95 and 100% DMSO caused greatly increased wheals and flares in wintertime, accompanied by a marked increase in Laser Doppler velocimetry. Conclusion: Even though the number of corneocyte cell layers increases in the winter, the above observations show that this is accompanied by impairment of the horny layer barrier. Histologic study reveals the presence of fine fissures and cracks in wintertime, increasing the permeability to water and exogenous substances.the horny layer barrier.

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Few Aspects of Disability of Children with Atopic Dermatitis

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Purpose: Little is known about the disability of children with AD. We prospective studed 326 children with AD, ages 5–8, middle index SCORAD 38.25 (from 21.25 to 68.52). Their fist complain was itching and discomfort, also; sleep desorders, headache, weakness, selfishness, low apettite. Recent studies showed a link between presence of AD and adaptation to school. Children with AD have low motivation to study compering to helthy children. All needed psycotherapeutic correction. Another aspect of disability low physical activity was diagnosed among 2/3 our patients. Most of them had problem with function of vegetative nervous system. Presense of deffect of external kind also lower the life quality and leads to social disability. Famales with children with AD had lower income and needed for help. Necessary aspect for good education is present for child in all classes, but our patient absent in schooll for many days. We compared condition children with AD were studed in rehabilition center, it is new health care system of treatment which includes treatment as well as social-pedagogical methods. and children with AD in public schools after two years. Children from fist group had middle index SCORAD 8.21 but from second one - 28.51. GPI was lower among children from public school. Therefo, we suggest that the prevention of disability as early as possible under and in elementary school children with AD are required.

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Characterization of Acute, Intermittent and Chronic Mouse Allergic Asthma Models

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Patients with allergic asthma present clinically with chronic or intermittent-seasonal disease, caused by either persistent or intermittent allergen exposure, respectively. To test novel treatment modalities during ongoing disease, we established two mouse models which more closely resemble clinical disease. To generate chronic and intermittent mouse models of allergic asthma, we immunized BALB/c mice with ovalbumin (OVA) (25 μg) in alum ip. on day 0 and 5 and nebulized with OVA (1%) 7 days later. We then evaluated the mice for methacholine induced airway hyperreactivity (AHR), lung inflammation (bronchoalveolar lavage fluid (BAL) and lung histology), mucus production and serum and BAL OVA-specific IgE and IgG1 either; 1.) at frequent intervals following sensitization or 2.) after once weekly OVA nebulizations. We observed increased AHR at 24 h and peak BAL eosinophilia 48 h after the last OVA aerosol challenge in sensitized mice compared to baseline responses in negative controls. By day 36 both AHR and BAL eosinophilia decreased to basal levels. However, when re-exposed to aerosolized OVA on day 41, mice developed increased AHR, BAL and lung eosinophilia that peaked 2 days later than acute disease. When we nebulized sensitized mice with weekly aerosolized OVA, we detected persistently elevated AHR, BAL and lung eosinophilia. We found increased mucus production in relapse and chronic compared to acute disease. All OVA-sensitized mice had elevated serum OVA-specific IgE and IgG1. In addition, we observed a remarkable increase in the BAL Ig during the relapse and then a steady increase in levels during chronic disease. Moreover, lung histology revealed an increase in plasma cells during disease relapse and a striking increase during chronic disease. In summary, we established intermittent and chronic mouse models of allergic asthma that differ from acute disease and are useful for testing novel treatment strategies.

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Confocal Histopathology of Irritant Contact Dermatitis, *In Vivo*: A Study of Ethnic Variability

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The pathogenesis of irritant contact dermatitis (ICD) is not well-understood. The goal of our study was to utilize *in vivo* confocal microscopy (CM), a noninvasive imaging technique that can optically section biological tissue with high resolution and contrast, to determine whether there is a difference in the susceptibility to ICD associated with ethnicity. Subjects were placed in groups based on ethnicity and then exposed to 1, 2, and 4% sodium lauryl sulfate (SLS) using the Finn Chamber method on inner-forearm skin. Each skin reaction was noninvasively assessed at 6, 24, and 48 h by *in vivo* CM, transepidermal water loss (TEWL), and laser Doppler velocimetry. Skin biopsies of the 4% SLS-treated sites were obtained for comparison. Irritancy threshold was found to be dose-dependent and related to ethnicity. In all subjects, CM revealed microscopic changes like stratum corneum damage as early as 6 h after SLS application, even without clinical evidence of irritation. Stratum corneum ablation was observed in caucasians at 24 h whereas only isolated areas of disruption were appreciated in black subjects. Although irritancy threshold varied between the groups, after 24 h there was a perivascular and epidermal inflammatory infiltrate, vascular dilation, blurred dermal appearance, and increased cell-to-cell demarcation. Routine histology supported the findings of CM. Ethnic variability was further supported with caucasians having greater TEWL rates than blacks. Our results demonstrate that *in vivo* CM can track pathophysiologic events revealing ethnic differences during the development of ICD.

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Imaged Perfusion is a Sensitive, Non-Invasive Method of Quantifying the Magnitude of Contact Hypersensitivity Responses in Humans

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One of the major deterrents to careful delineation of the immunological mechanisms involved in contact hypersensitivity reactions in humans has been the lack of a quantitative method with which to measure the magnitude of the response. In previous studies we have shown that Laser Doppler Perfusion Imaging (LDPI) is an effective noninvasive method of quantifying patch test reactions in humans that does not require contact with the skin. The objective of this study was to determine whether the magnitude of the perfusion of the contact hypersensitivity response as measured by the LDPI was associated with immunological parameters implicated in the pathogenesis of the disease. For this purpose, urushiol was applied under occlusion to test areas on one of the forearms of human volunteers for 48 h while the other forearm served as a control. Twenty-four hours later, measurements of perfusion of the patch test sites were performed with the LDPI technique. To determine whether there was a correlation with immunological parameters associated with human contact hypersensitivity, suction blisters were produced at the patch test sites. Blister fluid was removed and examined for the cytokine interleukin-8 (IL-8) by ELISA. There was an extremely close correlation between the magnitude of the contact hypersensitivity response as measured by the imaged perfusion and the level of IL-8 in the blister fluid ($r = 1.00$). Compared to subjects with visually positive urushiol reactions, patients who failed to develop urushiol contact hypersensitivity despite repeated exposures to that substance had both greatly diminished perfusion and blister fluid IL-8 levels. The results indicate that LDPI is a sensitive method of quantifying contact hypersensitivity reactions in humans and that the magnitude of the measurements with this technique correlates extremely well with cutaneous cytokine levels that have been implicated in the immunopathogenesis of contact hypersensitivity in humans.

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Clinical Characteristics and Outcome of Patients with Extracutaneous Mycosis Fungoides

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Purpose: To identify prognostic factors predictive of outcome in patients with extracutaneous (stage IV) mycosis fungoides (MF) and to evaluate the risk of progression to extracutaneous disease by initial extent of skin involvement. Materials & Methods: One hundred and twelve patients with extracutaneous disease at presentation or with progression and 434 patients with initial cutaneous-only disease were identified. Actuarial survival curves were plotted according to the Kaplan-Meier technique. Results: The median survival of all stage IV patients was 13 months from the date of first treatment for stage IV disease. Neither gender, race, age, extent of skin involvement, nor peripheral blood Sezary cell involvement was significant to survival outcome. Eleven patients (10%) had a complete response to therapy resulting in a significantly improved median survival compared to patients with a partial or no response (1.70 vs. 0.91 years, $p = 0.047$ and 1.70 vs. 0.57 years, $p = 0.011$, respectively). At 20 years from diagnosis, the risk for progression to extracutaneous disease by initial extent of skin involvement was 0% for limited patch/plaque, 10% for generalized patch/plaque, 35.5% for tumorous disease, and 41% for erythrodermic involvement. Conclusions: This was a larger scale study over a longer time period than had been completed previously on extracutaneous MF. Prognostic factors important in the cutaneous stages of disease are no longer significant once extracutaneous disease develops. Patients who had a more favorable response to therapy may have had a biologically less aggressive disease than their less fortunate counterparts. The risk of developing stage IV MF is highest in patients presenting with tumorous or erythrodermic skin disease and it is lowest in patients with limited skin involvement.

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Synergistic Enhancement of Cell-Mediated Immunity by IL-12 Plus IL-2: Basis for Therapy of Cutaneous T-Cell Lymphoma

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Cutaneous T-cell lymphoma (CTCL) is a clonally derived, skin-invasive malignancy of CD4+ T-lymphocytes with the phenotype of mature helper T-cells. Our previous work has demonstrated that the Sezary form, or typically leukemic form of CTCL, is characterized by prominent immunologic defects including depressed cell-mediated immunity associated with marked defects in production of IL-12 and other type 1 helper T-cell cytokines. Previous clinical trials with recombinant human IL-12 (rhIL-12) for CTCL have demonstrated that it is a potent therapeutic agent which induces cytotoxic T-cell responses. Nevertheless, a high rate of refractoriness to rhIL-12 occurred in these studies which may be related to the down-modulation of IL-12 receptor expression by chronic IL-12 use. In an effort to enhance the overall response rate and to overcome the refractoriness to rhIL-12 therapy, we studied the immunologic effects *in vitro* of adding IL-2 to IL-12 as a model to achieve these goals. We examined the stimulation of IFNγ production, natural killer cell activity and IL-12 receptor expression by T-cells of CTCL patients. The addition of IL-12 to CTCL patient peripheral blood cells resulted in the production of IFNγ (Mean = 27 pg per ml ± 13, n = 6) as did IL-2 alone (Mean = 70 pg per ml ± 30, n = 6). Importantly, the addition of IL-2 to the IL-12 synergistically enhanced the levels of IFNγ produced (Mean = 1373 pg per ml ± 435, n = 6) ($p < 0.01$). Similarly, addition of IL-2 to IL-12 synergistically enhanced both the NK cell activity of 6 CTCL patients as well as T-cell surface IL-12 receptor expression in comparison to the effects of IL-12 or IL-2 alone. Thus, IL-2 plus IL-12 unequivocally produces the synergistic enhancement of multiple parameters of cell-mediated immunity as well as up-modulating IL-12 receptor expression indicating that protocols combining these two potent immune enhancing cytokines may have added therapeutic benefit for CTCL.

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Tacrolimus Ointment in the Treatment of Chronic Cutaneous Graft-Versus-Host Disease: A Case Series of 18 Patients

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Tacrolimus (formerly FK506) is an immunosuppressive drug that works by inhibiting calcineurin, a calcium-dependent protein phosphatase required for immune function. Tacrolimus has been shown to be effective topically in atopic dermatitis, and systemically in psoriasis and graft-vs.-host disease (GVHD). However, its efficacy in treating cutaneous GVHD when applied topically has not been reported. The purpose of this case series was to assess the possible therapeutic efficacy of 0.1% tacrolimus ointment on chronic cutaneous GVHD which has been refractory to systemic corticosteroids. Effectiveness was measured by patient report, physical exam, side-to-side comparisons with a vehicle control, and temporal flares of the cutaneous symptoms of the disease in the context of stopping tacrolimus therapy. Tacrolimus ointment effectively treated pruritus and/or erythema in over 70% of 18 GVHD patients. Responding patients had a rapid effect within a range of several hours to days. However, because of progression of the disease and in two cases, loss of efficacy of the drug, all patients eventually went on to receive more aggressive treatment including an increase in steroid-dosing, psoralen and ultraviolet A (PUVA) therapy, and extracorporeal photopheresis (ECP). This case series suggests that tacrolimus ointment has efficacy in treating the erythema and pruritus of steroid-refractory cutaneous GVHD in a majority of patients. The rapid response of tacrolimus may provide a useful therapeutic bridge to systemic therapies that have a slower onset, such as PUVA therapy or ECP.

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Photopheresis Monocytes Produce Cytokines that Induce Monocyte-to-Dendritic Cell Maturation During Overnight Incubation

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A principal challenge for the development of an antitumor immune response is the loading of potent antigen-presenting cells, with tumor antigens for presentation in the context of accessory molecules necessary to initiate immune responses. Previous studies have shown that monocytes in the extracorporeal photopheresis (ECP) system become activated and differentiate after overnight incubation into immature dendritic cells (DC). We now demonstrate that inflammatory cytokines, derived from activated monocytes, are produced during the overnight incubation of the ECP product and may contribute to the observed rapid entry into the DC pathway. For analysis of the cytokine profile produced during the ECP procedure, specimens were obtained from blood and the 3 phases of the treatment: leukapheresis; 8-methoxypsoralen and ultraviolet A light exposure in the plastic plate; and overnight incubation. Cytokine levels were monitored using ELISA assays for:TNF-α; IL1-β; IL6; IFN-γ; IL4; GMCSF; IL10; and IL12. The results demonstrated that the overnight cultured ECP cells produced increased amounts of: TNF-α (3–10x); IL1-β (9–11x); and IL6 (6–11x) beyond that found in the pretreatment serum. No increase in the production of the cytokines: IFN-γ; IL4; GMCSF; IL10 and IL12 was found. The increased production of DC maturing cytokines, by activated monocytes during the overnight culture of ECP cells, suggests that these cytokines contribute to the rapid entry into the DC pathway. The presence of apoptotic tumor cells provides a source of tumor antigens for the differentiating DC and further drives their maturation. Thus, the cocultivation phase of the modified ECP treatment produces maturing DC loaded with the patient's unique tumor antigen repertoire, that is required for effective tumor vaccine immunotherapy, without prolonged culture, extensive manipulation or exogenous cytokine introduction.

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Narrowband Ultraviolet B Phototherapy for Early Stage Mycosis Fungoides

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Narrowband ultraviolet B (NB-UVB) phototherapy is an effective treatment for psoriasis and atopic dermatitis, but few studies have been published reporting the use of NB-UVB for mycosis fungoides (MF). This study was performed to evaluate the efficacy of NB-UVB in the treatment of MF. The records of 24 patients with IA (12 patients) and IB (12 patients) disease who received NB-UVB at Henry Ford Hospital between November 1998 and September 2000 were evaluated. There were 14 males and 10 females, all with patch stage MF; the mean age was 54.9 years (age range: 23–83 years), the mean disease duration was 5 years (range: 0.25–25 years), and mean follow-up period was 29 weeks. Twelve patients each had Fitzpatrick's skin phototypes I-III, and IV-VI, respectively. NB-UVB (TL-01 bulb) phototherapy was initiated three times weekly on nonconsecutive days. Minimal erythema dose (MED) determination was performed in all cases and the initial starting fluence was 70% of the MED. The fluence was increased by 10–15% increments each treatment. The physician's global clinical response to NB-UVB was: 13/24 patients showed = 95%clearing (complete response [CR]), 7/24 patients showed = 50% clearing (partial response [PR]), and 4/24 patients showed = 50% clearing (no response [NR]). Ten patients with CR had post-treatment biopsy, and all but one showed histologic clearance. Adverse effects were limited to erythema (42%) and phototherapy-associated pruritus (29%). This study indicates that NB-UVB is an efficacious and well-tolerated treatment option for patients with early stage mycosis fungoides.

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Identification of Amplified Clonal T Cell Populations in the Blood of Patients with Chronic Graft vs. Host Disease: Positive Correlation with Response to Photopheresis

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Chronic graft vs. host disease (cGVHD) is a major complication of bone marrow transplantation (BMT) and is responsive to photopheresis, a treatment that induces an anticlonotypic immune response and has proven to be effective in cutaneous T cell lymphomas with circulating clonal T cells. We have searched for circulating clonal T cell populations in patients with cGVHD, and determined whether T cell clonality in the blood is predictive of therapeutic response to photopheresis. Blood samples of 23 patients after HLA-matched allogeneic bone marrow transplantation (allo-BMT), 10 without cGVHD and 13 with extensive cGVHD, were screened for clonal T-cell receptor gamma gene rearrangements. Amplified clonal populations of T cells with unique TCR gamma gene rearrangements were found in 6 of 10 (60%) allo-BMT patients without cGVHD and 11 of 13 (84.6%) allo-BMT patients with cGVHD, as compared to 0 of 10 (0%) of healthy controls. Eight patients with cGVHD were treated by photopheresis, and the presence of amplified clonal populations of T cells was found to correlate with response to photopheresis, as 6 of 6 (100%) clone positive vs. 0 of 2 (0%) clone negative patients experienced a clinically significant response to treatment. A high proportion of patients with cGVHD have detectable expanded clonal T cell populations in their peripheral blood, and such patients may be more likely to respond to treatment by photopheresis.

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Bexarotene Combination Therapy for Cutaneous T-Cell Lymphoma

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Bexarotene (bex, Targretin(r) capsules) is an RXR selective rexinoid approved for all stages of CTCL with an overall response rate RR of 45% at 300 mg per m^2 per day. It is frequently used with statins or lipid lowering agents (LLA) for associated hypertriglyceridemia. Statins inhibit HMG-CoA reductase, modulate class II MHC expression, and reduce T-cell response (Nature Medical, December 2000). Bexarotene is now being combined with CTCL therapies, thus we evaluated 70 CTCL patients treated at MDACC. Fifty-four patients received bex with overall response rate (RR 48%), stage IA-IIA (n = 13, RR = 53%, 1 CR) and stage IIB-IVB (n = 41, RR = 46%, 2 CR). of these 54 pts, 41 (76%) received = 1 LLA: gemfibrozil (n = 3, RR 33%), atorvastatin (n = 27, RR 70%), or atovastatin/fenofibrate (n = 11, RR 81%). Advanced stage patients (15/16 erythrodermic) received bex at 225–750 mg per day in combination with ongoing therapy (interferon IFN, PUVA, photopheresis ECP, ONTAK) for an overall RR of 69% (11/16). Statins were given in 94% of them. Bex was combined with ECP (n = 6, RR = 66%), ECP/IFN (n = 4, RR = 50%), ECP/IFN/PUVA (n = 1, RR = 100%), ECP/ONTAK (n = 1, PR), PUVA/IFN (n = 3, RR = 66%) and IFN/PUVA/NM (n = 1, RR = 100%). Adverse events on combined therapy were limited to one idiosyncratic reversible neurologic event on bex/ONTAK and slightly low white counts in 2 patient on IFN. This single center CTCL study confirms the published response rate for bex used alone and supports safety of bex with other therapies to increase responses. Statins may augment therapy by allowing higher doses of bexarotene to be used or by favorably effecting the disease.

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Oral Bexarotene Therapy Combined with Phototherapy in the Management of Cutaneous T-Cell Lymphoma

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Oral bexarotene (BXT) has been found to induce responses in Cutaneous T-Cell Lymphoma (CTCL)patients at rates of 32–57%. We proposed that combinations of phototherapy and BXT could improve on monotherapy responses with either modality. Interactions of BXT with phototherapies for CTCL have not been observed. We report on 15 CTCL patients treated with oral BXT and phototherapy. The safety and efficacy of this combination therapy was recorded. A total of 15 patients used a combination of a phototherapy (8 with extracorporeal photochemotherapy (ECP), 6 with photochemotherapy with UVA light (PUVA), and 1 with (UVB)with oral BXT for 3 months or greater. Skin scores and serum tests for BXT toxicity were monitored. Eight patients with Sezary syndrome and partial responses to ongoing ECP had oral BXT therapy initiated at 300 mg per M2 (normal weight) or 150 mg per M2 (obese). After a minimum of 12 weeks of combination therapy, 3 had complete clinical responses, 4 had 50% improvement of body surface area involvement, and 1 had no response. Four of the Sezary syndrome patients had elevated lymphocyte counts with elevated (6.0)peripheral blood lymphocyte CD4:CD8 ratios that did not change as the skin cleared. The initial four patients treated with PUVA and oral BXT had partial responses to PUVA. In these patients, oral BXT was started at 150 mg per M2 and UVA doses were reduced 10%. Three patients had a complete clinical response, one had a reduction of body surface area 50%. Three patients with risk factors for PUVA failure (histologic variants of CTCL) were started with oral BXT (150 mg per M2) at the time phototherapy was initiated. All three patients had phototherapy doses safely advanced with minimal phototoxicity (two with PUVA, one with UVB. All three had complete clinical responses. Adverse events noted were dose responsive with BXT. Of the eight Sezary syndrome patients, 3 required antilipemic agents and 5 were given thyroid supplementation to counter the effects of oral bexarotene. In the 7 patients with PUVA or UVB the lower dose regimen of BXT required antilipemic therapy in one patient and thyroid supplementation in two. We conclude that oral BXT therapy can safely and effectively be used in combination with phototherapies in the management of CTCL. No adverse interactions of oral BXT were observed in patients treated with ECP, PUVA or UVB. These findings suggest that oral BXT therapy may have a role as a concomitant agent in the phototherapy of patients with CTCL.

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Natural Killer (NK)/NK-Like T-Cell Lymphoma (CD56+) Presenting in the Skin: An Increasingly Recognized Entity with an Aggressive Course

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Objective: To describe and identify the clinical and pathologic features of prognostic significance for NK/T-cell lymphoma presenting in the skin. Methods: A retrospective analysis of 34 pt with CD56+ lymphomas initially presenting with cutaneous lesions with analysis of clinical and histopathologic parameters. Results: The med survival for all pt is 15 months. Pt with no extracutaneous (EC) dz (n = 20; localized stage I_E = 8, disseminated skin IV_{D+M-} = 12) on intial staging evaluation have better survival rates at 1 yr (88%) and 3 yr (70%) compared to those with EC dz (n=12) with 16% alive at 1 yr and with no survivors at 3 yr ($p<0.0001$). Age, gender, extent of skin involvement, and initial response to therapy have no significant effect on survival. 22/34 (65%) stain with antibodies against CD3ε. 7/24 (29%) demonstrate clonal rearrangement of TCR-γ. 8/30 (26%) patients with unequivocal EBV results have detectable EBV within neoplastic cells. Rates of EBV+ tumors by pt country of birth: U.S 3/25 (12%), Asia/Pac.Island 2/2 (100%), Central/S.Amer 2/2 (100%), India 1/1 (100%). 22/29 patients (76%) evaluated have multi-drug resistant (MDR) gene expression. Pt with tumor cells that co-express CD30 (n=8) have better survival rates at 1 yr (100%) and at 3 yr (86%) compared to those with CD30- tumor cells with survival rates of 39% at 1 yr and 17% at 3 yr ($p=0.002$). Routine histologic characteristics have no prognostic predictive value and no outcome difference is found for those with tumor cells that are CD3ε+ ($p=0.8$), EBV+ ($p=0.3$), MDR+ ($p=0.19$). Conclusions: NK/T-cell lymphoma presenting in the skin is an aggressive neoplasm. A subset of pt with a more favorable outcome is identified in this study. The presence of EC dz at presentation is the most important clinical variable and portends a poor prognosis. The extent of initial skin involvement does not reliably predict outcome. As opposed to findings from countries where early infection with EBV is more common, pt from the U.S. with NK/T-cell lymphoma presenting in the skin have a low incidence of demonstrable EBV in their tumor cells. Pt with co-expression of CD30 in CD56 lymphomas tend to have a more favorable outcome than those with CD30- NK/T-cell lymphomas. However, further studies are needed to confirm the impression.

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Cutaneous B-Cell Lymphoma (CBCL): Superior Prognosis for Patients Presenting with Disease Limited to the Skin

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Purpose: Identify prognostic clin features for pt with CBCL. Patients and Methods: Retrospective analysis of 107 pt with B-cell lymphoma presenting in the skin, single institution. Results: Classification: 47 diffuse large cell (DLCL), 30 follicular (FL), 26 marginal zone (MZL) lymphoma, 3 other. Med follow-up is 56 mos. For DLCL, med age is 60, 31M/16F. Stages are I_E = 23, II_E = 5, IV = 19. The 4-yr OS for DLCL is 72%. Dz specific survival (DSS) is 75%. At presentation, 30 (68%) pt had dz limited to skin, 14 had extracutaneous dz (EC). Of pt with dz limited to skin, 23 are local (I_E), 10 are dissem (IV_{D+M-}). 4-yr DSS for I_E is 94% and vs. 50% for st II_E-IV with EC dz (n = 14; $p < 0.002$). 4-yr DSS is similar for st I_E and IV_{D+M-} (94% vs 80%; $p = 0.3$). DSS is not affected by age or type of tx. 32/33 pt with DLCL limited to skin had CR to initial tx and 17/32 (53%) relapsed (med 12 mos). Relapse risk by tx is chemo 5/12 (42%), XRT 6/12 (50%), chemo/XRT 4/7 (57%), other 2/2 (100%). Site of 1st relapse: skin only 13/17 (76%), other extranodal site 3/17 (18%), skin & LN 1/17 (6%). 13/15 (86%) had 2nd tx with a 2nd CR. 12/15 (80%) remain AIR. Characteristics for low grade (LG): FL med age 54, 22M/8F, MZL med age 52, 9M/18F. Pt with LG lymphoma limited to skin (I_E or IV_{D+M-}, n = 37) have a 4-yr DSS of 100% compared to 80% for st II_E-IV with EC (n = 21; $p < 0.001$). For st I_E (n = 31) and IV_{D+M-} (n = 6) tx are XRT 22,chemo 3, other 12. 16/36 (44%) relapse at a med of 11 mos (1-120). In 14/16 relapse is confined to skin. Conclusions: Pt with CBCL limited to the skin at presentation (I_E or IV_{D+M-}), have a superior prognosis compared to those with EC dz. Pt with EC dz at presentation have OS/DSS comparable to nodal lymphomas. Among those with I_E or IV_D limited to skin, DSS and risk of relapse are not affected by tx type. 82% of relapse is confined to the skin and responds well to further tx.

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Skin Cancer: Epidemiological and Clinical Aspects

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The studies purpose is to present several epidemiological and clinical characteristics of patients with Skin Cancer diagnosed in a Dermatology Clinic in Albania. We reviewed the clinical records of the 48 cases admitted in the clinic of dermatology at UHC of Tirana during 5-year period with the Skin Cancer. The inclusion criteria was considered the results of skin biopsy positive for skin cancer. The factors included in the study were:histopatological charasteristics, sex, age, risk factor, localization. There were 48 cases of skin cancer diagnosed at the clinic of Dermatology during a 5-year period, 38 cases (79.2%) were male and 10 cases (20.8%) were female. According to histopatological findings there were 25 cases (52%) with BCC and 23 cases (48%) with SCC. According to localization of the skin cancer there were 17 cases (35%) with the localization at the lower lip, 7 cases (14.5%) at the dorsum of the nose,6 cases (12.5%) at the ear and 9 cases (18.7%) localized at the rest of the face. Skin cancer predominates in male patients. Conclusion: There was not significant difference between cases with BCC and SCC among our patients. The ultraviolet sun exposure was seen as the main risk factor for cases with skin cancer. The lesion were localized mostly in lower lip and other areas exposed more at the sun light.

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Cost of Nonmelanoma Skin Cancer in the United States – Treatment in Physicians' Offices is More Common and More Cost Effective

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Background: Despite being the most prevalent form of cancer, the economic impact of non-melanoma skin cancer (NMSC) in the United States has not been assessed. Objective: The purpose of this study was to determine the overall cost and cost per episode of care of NMSC in the United States in physicians' offices, outpatient surgical center, and inpatient settings. Methods: Data from the Medicare Current Beneficiary Study 1992-1995 were analyzed to obtain the total cost of NMSC and the cost in different settings. To normalize these data to a per episode basis, the cost in each setting was divided by the number of procedures performed in each setting obtained from the National Hospital Discharge Survey (NHDS, 1992-1997), the National Survey of Ambulatory Surgery (NSAS, 1994-1996), and the National Ambulatory Medical Care Survey (NAMCS, 1995). Results: Physician office based procedures for NMSC accounted for the greatest percentage of money spent to treat NMSC and the greatest percentage of procedures. The average cost per episode of NMSC when performed in a physician's office setting was found to be $492. The cost per episode of care in inpatient and outpatient settings were $5537 and $1043 respectively. Conclusion: Physician office-based treatment of NMSC is far more common and significantly less expensive than outpatient or inpatient care. Legislative or regulatory measures that discourage office treatment of skin cancer will lead to increased cost and poorer outcome.

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Laws Regulating Tanning Booth use by Minors

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Ultraviolet (UV) light is a carcinogen. Epidemiological data demonstrates that UV irradiation, especially during childhood, is a risk factor for skin cancer and melanoma. Because greater than 80% of lifetime UV irradiation is estimated to occur before age 20, the behavior of minors with respect to tanning is a prime target for skin cancer prevention, and restriction of tanning facility use by minors may be a justified public health intervention. For example, requiring all minors to obtain parental consent prior to patronizing tanning facilities would better assure thoughtful consideration of short-term gains, i.e. temporary increased physical attractiveness, vs. long-term risks, i.e. increased skin cancer, and might decrease the number of minors engaging in this carcinogenic behavior. The vast majority of US states have no age-specific restrictions governing access to tanning salons. Wisconsin is the only state with an age-specific requirement for those seeking tanning services; tanning booth use is restricted to those age 16 and older. Only seven US states (FL, IN, LA, MA, MI, TN, TX) require parents to accompany children under age 14 to tanning booth facilities (Indiana's parental companion restriction, being more extensive, applies to minors under age 16). And only 10 states (CA, GA, FL, IN, LA, MA, MI, MN, TN, TX) require written parental consent from minors in their late teens seeking tanning services. Regional variation in the regulation of minors using tanning parlors may provide an opportunity to measure the effect of legal restriction on the tanning behavior of US minors. This study further compares US age-specific tanning booth regulations to those of other nations, and to age-specific regulations on other carcinogenic behaviors, e.g. cigarette smoking. To further this comparative summary, SID meeting participants are asked to inform the presenters of relevant laws in their native lands.

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Nonmelanoma Skin Cancer in Persons with Prior Cutaneous Melanoma

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Background: Melanoma has been associated with an overall increase in actinic tumors, including actinic keratoses, as well as with non-cutaneous malignancies. Objective: Determine the risk of developing basal cell and squamous cell skin cancer in patients with prior cutaneous melanoma (actinic keratoses not encountered). Methods: This retrospective study included 1396 white persons with prior cutaneous melanoma followed at the Roswell Park Cancer Institute in the period 1977-1978. The control group was the white population of the Detroit area in the same period (1977-1978). Results: Totally, 25 patients (18 males/7 females) developed 35 basal cell and/or squamous cell carcinomas of whom 18 developed basal cell carcinoma(s), 2 squamous cell carcinoma(s) and 5 both. The calculated odds ratio was 3.49 (males 3.67, females 2.86, 95% confidence interval 1.52-8.00). No correlations were found with age, type, anatomic site, and length of follow-up of cutaneous melanoma. Conclusion: History of cutaneous melanoma significantly increases the risk of basal cell and squamous cell skin cancer.

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How do People Apply Sunscreen?

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Broadband sunscreen agents are effective against sunburns, UV-induced immunosuppression as well as UV-induced DNA damages, suggesting their potential protective effect in skin carcinogenesis. However, controversial hypotheses were raised concerning a potential link between use of sunscreens and the risk of melanoma. Controlled prospective studies are hindered by the difficulties in quantifying UV exposure and sunscreen product use over a long period of time. We studied a critical parameter of sunscreen-mediated photoprotection, i.e. the quantity of product applied and the quality of sunscreen spreading on the skin by 17 female volunteers. The women were asked to spread a sunscreen (SPF 30) exactly as they would before sunbathing. The sunscreen was visualized using UVA light and photos of the entire body were taken. The quantity of sunscreen applied by each volunteer was calculated and the spreading efficiency was evaluated on the photos by three independent investigators using an intensity visual scale. Our results show that the mean total quantity of sunscreen agent applied is very low and represents on average only 27% of the quantity required to realize the labeled SPF. Furthermore sunscreen spreading was very heterogeneous. We provide a sunscreen spreading cartography of the human body and show that certain skin surface areas were reproducibly forgotten and left unprotected. We show here that spontaneous sunscreen self-application is quantitatively insufficient and qualitatively poor which obviously hinders photoprotection. This might represent a major bias in epidemiological studies and explain, at least in part, the risk of sun damage in people who think they are protected. To reduce this risk, we propose here an optimized method of sunscreen body application and spreading. We think this is an important public health message that should be brought to public awareness as much as SPF and UVA protection.

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Lifestyle High-Risk Behaviors and Demographics may Predict the Level of Participation in Sun Protection Behaviors and Skin Cancer Primary Prevention in the United States

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Background: Sun and UV radiation exposure are major risk factors for skin cancer. Sun protective behaviors and skin cancer exams are means of primary prevention of skin cancer. Objective: The purpose of this study was to evaluate the extent to which demographics and other high-risk lifestyle behaviors may predict the reported level of participation in sun protection behaviors and skin cancer primary prevention in the United States adult population. Methods: Data on reported sun protection behaviors and skin cancer examinations was obtained from surveys completed by adults in the 1998 National Health Interview Survey. Univariate and multivariate data analyses were performed using Stata software. Results: For the United States adult population surveyed ($n = 32,440$), only 21% indicated that they had ever had a skin cancer exam, and of those, only 45% indicated that the skin cancer exam was within the past year. For sun protective behaviors, only 23%, 27% and 30% of those surveyed reported to be "very likely" to "wear protective clothing", "stay in the shade" and "use sunscreen" respectively. Likelihood of participation in sun protective behaviors and skin cancer prevention was affected by demographic characteristics such as gender, race, age, education level, income level, region of the country, marital status and metropolitan area size. In addition, lifestyle high-risk behaviors, including duration since last general physical examination, currently smoking cigarettes, wearing of a seatbelt when riding in the front seat of an automobile and possession of firearms appeared to impact the reported level of participation in sun protective behaviors. Conclusions: A number of demographic factors and lifestyle high-risk behaviors may predict the likelihood of participating in sun protective behaviors in United States adults.

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Phase III Clinical Study Demonstrating Prevention of Skin Cancer in Xeroderma Pigmentosum by Topical Application of T4N5 Liposome Lotion Containing DNA Repair Enzymes

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Patients with xeroderma pigmentosum (XP) have an elevated incidence of skin cancer resulting from genetic defects in DNA repair. Intracellular delivery of the bacterial DNA repair enzyme, T4 endonuclease V, increases the rate of repair of sunlight induced DNA damage in human cells. Therefore, we tested the ability of this DNA repair enzyme in a liposomal delivery vehicle applied topically (T4N5 liposome lotion) to reduce the incidence of skin cancer in XP patients. This was a prospective, multicenter, placebo-controlled, randomized and double-blinded study on the incidence of new skin cancer and actinic keratosis (AK) in 30 XP patients applying T4N5 liposome lotion daily for one year. The annual rate of new basal cell carcinoma (BCC) was 5.4 cancers per patient per year in the placebo group and 3.8 in the T4N5 Liposome Lotion group. This represents a 30% reduction ($p = 0.006$, Poisson regression). Furthermore, the annual incidence rate of AKs was 25.9 lesions per patient per year in the placebo group and 8.2 in the T4N5 Liposome Lotion group. This was a 68% reduction ($p = 0.004$, Poisson regression). No significant adverse effects were found among any of the patients. DNA damage plays a powerful role in the development of both skin cancer and precancerous skin lesions. The topical application of liposomal DNA repair enzymes to sun damaged skin of XP patients reduces the incidence of at least some forms of these lesions, namely BCC and AK, within one year.

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Tazarotene Reduces BCC Tumor Number and Size in UV Exposed ptc+/– Mice

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Oral retinoid treatment reduces basal cell carcinoma (BCC) incidence in patients with basal cell nevus syndrome (BCNS) (Peck *et al* 1988). Because extended tazarotene gel application results in regression of 53% of sporadic BCCs (Peris *et al* 1999), we sought to determine whether oral tazarotene would have a comparable or superior effect in ptc+/– mice that, like BCNS patients, have an inactivating mutation of one patched allele. Also like BCNS patients, ptc+/– mice develop few, microscopic BCC-like tumors on UV-protected skin, and when exposed to ionizing radiation or UV, develop numerous and significantly larger tumors. Ptc+/– mice were exposed to tumorigenic doses of UVB for 7 months, irradiation was stopped, and mice were randomized to receive vehicle or oral tazarotene at 1, 2.5, or 5 mg per kg ($n = 18$ per treatment arm) five times per week. Skin biopsies from UV-exposed sites were taken 30, 60 and 120 days after therapy was initiated. After 60 days of treatment, the average tumor size in all tazarotene treated groups was 50% less than in controls (0.01 vs. 0.02 mm^2; $p < 0.002$ using student's t test). However, the average tumor number was not significantly reduced by tazarotene treatment at this time point. After 120 days of treatment, animals receiving 1 and 5 mg per kg tazarotene had 60 and 40% fewer tumors vs. controls (5.73 and 7.56, respectively, vs. 13.8 tumors in controls; $p < 0.002$, $p < 0.023$). Animals treated with 2.5 mg per kg had a modest but not statistically significant reduction in tumor number (10.6 vs. 13.8 tumors). Moreover, the average tumor size in all treatment groups was not reduced at 120 days. We conclude that ptc+/– mice treated with oral tazarotene have a comparable tumor regression rate (approximately 50%) as humans treated with topical tazarotene. This mouse model may help elucidate the mechanisms underlying tazarotene's chemotherapeutic effect.

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Clinical and Immunologic Response of Extramammary Paget's Disease to Imiquimod

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In an unlabeled use of a commercial product 1% imiquimod cream had a good effect in treatment for extramammary Paget's disease (~ 14×23 cm^2 area). Nine years previously the 84 years male had bright erythema of the urethral meatus, crural folds and scrotum with maceration and discharge was clinically diagnosed as yeast and contact dermatitis and treated with ketoconazole orally (200 mg per day) for 5 days and topical steroid and 1% econazole cream. The topicals were continued. Six months ago, when the tumor diagnosis was made, his symptoms had progressed to include discouragement, pain, and decreased ability to walk and signs – enlarged red plaques and papules covered with blood tinged, purulent, and malodorous discharge – which involved the urethral meatus, parts of the shaft of the penis, scrotum, pubic area, inner thigh and buttock. Initial histology showed classical features of extramammary Paget's disease. The epidermis was nearly effaced by a population of plump round cells with enlarged hyperchromatic nuclei and occasional intracytoplasmic lumina. There was increased mitotic activity and individual cell necrosis. The eccrine glands were also extensively infiltrated. Paget's cells were positive for CAM5.2, EMA, mucicarmine and pan-cytokeratin. S-100 was negative. No underlying malignancy was found. The patient was treated with 1% imiquimod cream applied to all tumor for 3 months. Within a month pain and foul discharge were markedly improved. At three months all pain, discharge was gone. Clinically there was the appearance of a postinflammatory erythema and microscopically a complete histologic remission of Paget's disease. There was, however, a lichenoid dermatitis with eosinophiles most consistent with a drug eruption. The response did not include all areas. Unresolved smaller plaques of Paget's disease (~ 2×3 cm^2) were treated with CO_2 laser ablation.

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Inhibition of DNA Methylation by Continuous Infusion of 5-Aza-2′-Deoxycytidine (Decitabine): A Phase I Study with Molecular Goals

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One proposed mechanism of action of the deoxycytidine anaolog, 5-Aza-2′-Deoxycytidine (Decitabine), is through the marked reduction in cellular DNA methylation and subsequent gene reactivation. Decitabine has been used to treat patients with leukemia, but has no proven efficacy in solid tumors. This may be due to inadequate incorporation of the drug into slowly dividing solid tumor cells (because the drug has a short half-life and is usually administered in a bolus fashion). Therefore, we have designed a phase I study with the following primary objectives: (1) establish the feasibility of measuring DNA methylation changes, gene induction, and proliferation status in the blood and epidermis of patients before and after treatment (2) determine the maximum tolerated dose of continuous i.v. Decitabine infusion over 7 days and (3) identify the dose-limiting toxicities of a 7-day Decitabine infusion. Our basic science studies are designed to determine the biologic optimum of the drug. Preliminary *ex vivo* experiments, have been proceeding in parallel with the development of the clinical trial. We have utilized a skin explant model to optimize protocols for DNA methylation and methylation-induced gene activation in clinical samples. Western blot and immunohistochemical protocols to evaluate several methylation sensitive genes (MHC class I, STAT1, STAT3, and transglutaminase I) have been developed. Mitotic index is being evaluated using topoisomerase II in a well-established semiquantitative assay. Preliminary data from these experiments suggest that it will be feasible to determine basic scientific endpoints in clinical samples. This protocol translates our understanding of the molecular action of Decitabine into a clinical trial that tests this mechanism *in vivo*.

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Imiquimod 5% Cream is Safe and Effective in the Treatment of Actinic Keratosis

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Actinic keratosis (AK) is the earliest clinical manifestation of squamous cell carcinoma. To determine the efficacy and safety of imiquimod 5% cream as a treatment for AK. Twenty-two patients with AK were treated with imiquimod 5% cream, initially at three times a week, for eight weeks or until clearance of lesions. Patients applied imiquimod to lesions on one side of the body and vehicle cream to the other side. Seventeen patients were evaluated for total number of lesions and adverse reactions before treatment and at weeks 2, 4, 6, and 8, then at 4 and 8 weeks post-treatment. A significant reduction in the average number of lesions per patients was observed for imiquimod-treated patients. The majority of patients experienced mild to moderate adverse events. The two most frequent reactions were irritation and reddening of the skin. Imiquimod 5% cream is a promising effective treatment of actinic keratosis with an acceptable safety profile.

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Photodynamic Therapy (PDT) with Methyl 5-Aminolevulinate 160 mg per g Cream in Patients with Basal Cell Carcinoma (BCC) with a Risk of Complications and Poor Cosmetic Outcome Using Conventional Therapy

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Patients (pts) with ''high-risk'' BCC in need of advanced surgery or radiation therapy with a risk of complications and poor cosmetic outcome, received a new selective photosensitizer, methyl 5-aminolevulinate (MAL) to determine response rate, cosmetic outcome and side effects. Pts with clinical and histological diagnosis of BCC (mid-face, large, recurrent) excluding morpheic and highly infiltrating lesions, received one treatment cycle with MAL PDT (two treatments one week apart). After lesion preparation and 3 hrs of topical occlusion with MAL cream (Metvix®), the lesion was illuminated with 75 J per cm^2 of red light (570–670 nm). If there was non-complete response after 3 months as assessed clinically and by histology, the lesion was retreated. Ninety-four pts with 123 lesions were treated and included in safety analysis. Sixty percent of the lesions were located in face/scalp; 40% of the patients received two treatment cycles. Eighty-five pts with 108 lesions were included in primary efficacy analysis, nine pts were excluded by external reviewer because they did not fulfill the definition of having a ''high-risk'' BCC lesion. Clinical lesion evaluation resulted in complete response rate of 87%, which dropped to 74% when excluding lesions with a positive histology. Seventy-five percent of pts had good or excellent cosmetic outcome. Sixty-seven percent of pts reported adverse events, mostly expected local phototoxic reactions like erythema and burning sensation/pain. The symptoms were transient, and mostly of mild severity. MAL PDT was effective in pts with ''high-risk'' BCC and the cosmetic results were mostly good or excellent. MAL PDT was well tolerated and may be a good alternative to conventional modalities which have the risks of disfiguration and inferior cosmetic outcome. Follow-up is underway to determine long-term recurrence rate. *Other authors: L. Rhodes, C. Fritsch, R. Kaufmann, M. de Rie, I. Stender, A. Solér, A.M. Wennberg, G. Wong, F. Legat, S. Pavel; Univ. of Liverpool, UK, Heinrich-Heine Univ. Düsseldorf, Germany, Goethe Univ. Frankfurt, Germany, Academic Medical Center Amsterdam, The Netherlands, Leiden Univ. Medical Center, The Netherlands.

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Arsenic Trioxide Induces a p53-Dependent Apoptosis in Skin Cancer Cells

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Arsenic trioxide (As_2O_3) was recently found to induce complete remission in the patients with refractory acute promyelocytic leukemia (APL) and to inhibit proliferation and induce apoptosis in the APL cell line NB4. Afterwards, As_2O_3 was reported to have cytotoxic effects in several human cancers including solid tumors by induction of apoptosis. We wondered whether As_2O_3 was able to induce apoptosis in skin cancer cells. We demonstrate in this report As_2O_3 induces apoptosis in skin cancer cell lines such as human basal cell carcinoma (BCC), human epidermoid carcinoma (A431), human malignant melanoma (Hs695T) and PRMI 7951 in a dose- and time-dependent manner, as evidenced by internucleosomal DNA fragmentation and morphologic changes. By Western blot analysis, we found that the induction of apoptosis involved an early increase in p53 protein and caspase 3 activation; however, the expressions of Bcl-2 and Bax were not changed after the treatment of As_2O_3. In addition, pretreatment of these skin cancer cells with p53 antisense oligonucleotide could effectively block As_2O_3-induced apoptosis, but not by p53 sense oligonucleotide. Thus our findings suggest that the p53-associated signaling pathway is critically involved in As_2O_3-mediated apoptotic cell death.

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Sequence of Events During Photodynamic Therapy-mediated Apoptosis of Human Epidermoid Carcinoma A431 Cells

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Photodynamic therapy (PDT) is a cancer treatment modality, which takes advantage of preferential accumulation of porphyrin-based photosensitizers in tumor as compared to normal tissue followed by selective delivery of visible light to the target tissue. This results in the activation of the photosensitizer that causes oxidative damage leading to tumor ablation. PDT has emerged as a novel treatment modality in dermatology inasmuch as it is showing promise for skin cancer and several nonmalignant skin conditions. Recently PDT has received approval for the treatment of actinic keratoses. PDT has been shown to cause oxidative stress leading to the killing of cells via apoptosis. An understanding of molecular events in PDT-mediated apoptosis is not completely understood; unraveling these pathways could lead to improvement in therapeutic efficacy of PDT. We have earlier shown the involvement of (i) Fas-FADD-FLICE and (ii) bcl2, bax and other bcl2 family proteins during silicon phthalocyanine (Pc 4)-PDT-mediated apoptosis of human epidermoid carcinoma A431 cells. Extending this work, here we show the involvement of cytochrome c-Apaf-1-caspases as the downstream events in this apoptotic-pathway. Immunoblot analysis of cells photosensitized with Pc 4-PDT showed a rapid release as early as 1 h post-PDT of cytochrome c from mitochondria into the cytosol at which time only less than 7% of cells were apoptotic. This was followed by a significant increase in (i) the protein expression of Apaf-1 (apoptosis inducing factor-1) (ii) caspase activity (as assessed by the release of AFC from DVED-AFC substrate by fluorescence spectroscopy), and (iii) the protein expression of caspase-3, –7, –8 and –9, at 3 h (12% apoptotic cells) and 6 h (21% apoptotic cells) post-PDT. Further, PDT of cells also resulted in a gradual loss in the protein expression of DNA fragmentation factor (DFF) and an increase in the cleavage of PARP in a time-dependent fashion. Based on these data, we suggest that Pc 4-PDT of A431 cells results in Fas-FADD-mediated loss of bcl2 with an increase in bax, which leads to a mitochondrial dysfunction causing a release of cytochrome c from the mitochondria into cytosol. Once released into cytosol, cytochrome c binds to its adaptor molecule, Apaf-1, which oligomerizes and activates caspases that, in turn, results in cleavage and activation of DFF leading to PARP cleavage. This series of events ultimately leads to an apoptotic death of cancer cells.

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Increase of Mortality Rate by Malignant Melanoma in Chile

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The purpose of the study is to assess the impact of malignant melanoma in Chile and to evaluate a long-term trend of its frequency. Mortality rates by all skin cancers and by malignant melanoma were calculated from 1970 to 1998. In Chile in 1998 the mortality rate by all skin cancers was 1.55 per 100,000, with 230 deaths; mortality rate by melanoma was 0.85 per 100,000, with 126 deaths. This mortality rate represents a 400% increase as compared with the rates from 1970. There were no major differences in mortality rates according to gender. Mortality rates by all skin cancers increased dramatically in patients older than 75-year-old. In conclusion, this study revealed a substantial increase in the last 30 years in mortality rates by malignant melanoma in Chile.

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Do Lipid-Lowering Medications Prevent Melanoma?

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Two randomized, double-blinded, placebo-controlled, five-year trials of lipid-lowering agents have revealed significantly lower melanoma rates in treated patients than in patients receiving placebo. One trial (the VA-HIT study) utilized a member of the fibrate drug class, gemfibrozil, and the other trial (the AFCAPS study) utilized a member of the statin drug class, lovastatin. Interestingly, lovastatin has previously been shown to inhibit melanoma cell growth in tissue culture, and to decrease melanoma metastasis in mice injected with melanoma cells. The hypothesis that lipid-lowering medications prevent melanoma was explored via a case-control analysis of the Denver Veterans Administration Medical Center (VAMC) diagnostic and pharmaceutical databases. 328 melanoma cases were identified (64 in-patients from 1986 to 2000 and 264 out-patients from 1996 to 2000) along with a random sample of two thousand controls. To assure that controls represented active patients, controls were chosen from a pool of patients who had filled at least one prescription for any medication from July–August 1999 and again from July–August 2000. The exposure rate of any statin prescription filled at the Denver VAMC pharmacy from 10/1/92 to 9/30/00 was 18% among cases and 30% among controls. The exposure rate for fibrates was 4.9% among cases and 5.9% among controls. Further characterization of the temporal sequence and size of exposure is needed before these preliminary results (lower prescription fill rates of lipid-lowering drugs in cases) can be said to support the hypothesis that lipid-lowering medication prevent melanoma. Still lipid-lowering medications may provide a novel method of modifying melanoma risk. Further investigation of lipid-lowering medications for the chemoprevention of melanoma will determine whether randomized trials of these agents should be initiated in persons at high risk of developing melanoma.

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A Population-Based Study of Gender Differences in Melanoma Epidemiology: 1988–97

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To date, gender differences in melanoma (MM) epidemiology have not been fully elucidated. We hypothesized that there are distinct age-specific incidence patterns for MM in males and females. Subjects included 10 489 primarily non-Hispanic whites with MM analyzed from population-based cancer registries of three Southern California counties, 1988–97. For further analysis of age- and gender-specific incidence, we also evaluated 39 806 subjects from the entire California Cancer Registry, 1988–97. The incidence of MM increased with increasing age during the study period for both men and women, except in perimenopausal women aged 45–60, during which time the incidence remained constant. The incidence of MM in subjects less than 40 in both genders was roughly equal. After age 40 the incidence of MM increased with age considerably faster for males. MMs in both genders increased dramatically during the study period, due primarily to localized, thin tumors. The incidence in males increased at an annual rate of 4.4%, but 5.5% in females ($p < 0.0001$). Melanoma *in situ* increased at a rate of 15% per year. In a multivariate analysis the following variables, but not gender ($p < 0.906$), were associated with worse survival: increasing tumor thickness ($p < 0.0001$), increasing age ($p < 0.0001$), truncal vs. limb ($p < 0.0001$) or head/neck ($p < 0.016$) location, and nodular melanoma vs. all other histologic types ($p < 0.0001$). Survival did not change over the study period. In this population-based study, we note for the first time a striking female perimenopausal plateau in the age-specific incidence of MM. The cause of this perimenopausal plateau is unknown, but could include a transient hormonal or other menopausal influence, the waning of a premenopausal hormone, or a cohort-specific effect.

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Pilot Study of the Diagnostic Accuracy of Patients with Dysplastic Nevi in Performing Skin Self-Examination (SSE)

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We conducted a pilot study to determine the sensitivity and specificity of SSE to detect new and changing moles, with and without the aid of baseline digital photography, in patients at high risk for skin cancer. Patients with 5 or more dysplastic nevi were recruited from the outpatient clinic at Memorial Sloan-Kettering Cancer Center. Patients had baseline digital photography and mole counts of their back and abdomen and were instructed to perform a baseline SSE. This was followed by the alteration of existing moles and the creation of new moles with the use of body paint. Each patient had approximately 3 mol altered and/or created. Blind-folding of patients and sham drawing on multiple sites were used to ensure that the patients were unaware of the location of cosmetically altered moles. Patients were then asked to perform SSE first without the aid of baseline photographs and subsequently with access to photographs. Data were recorded for the number of new and altered moles correctly and incorrectly identified by the patients and the sensitivity and specificity of SSE (using the mole as the unit of analysis) were calculated. Mole counts on 45 patients provided data on 2957 mol. A total of 101 and 197 mol were altered and created, respectively. The sensitivity and specificity of SSE for detection of both altered and new moles without photography (WOP) were 59.7% and 96.5%, respectively. SSE with photography (WP) yielded a sensitivity and specificity of 73.2% and 98.6%, respectively ($p < 0.0001$ for paired comparison of SSE tests). For the back only, the sensitivity of SSE for detection of altered/new moles was 57.1% (WOP) and 69.3% (WP) ($p < 0.0001$). For the abdomen only, the sensitivity of SSE was 64.2% (WOP) and 79.8% (WP) ($p < 0.0001$). The sensitivity of SSE for detection of altered moles was 53.5% (WOP) and 65.3% (WP) ($p = 0.001$). The sensitivity of SSE for detection of new moles was 62.9% (WOP) and 77.2% (WP) ($p < 0.0001$). No important differences were observed for specificity in the stratified analyses. Access to baseline photography improved the diagnostic accuracy of SSE on the back and abdomen and improved detection of changing and new moles. Our results suggest that baseline digital photography in tandem with SSE may be effective in improving the diagnostic accuracy of SSE.

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Bryostatin Potentiates the Susceptibility of Human Melanoma Cell Lines to Fas-Mediated and Cisplatin-Induced Apoptosis

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Melanoma cells maintain a wide-variety of antiapoptotic defenses which limit the effectiveness of chemo-, immuno-, and radiotherapy designed to induce apoptosis. Bryostatin also shows limited effectiveness in single agent therapy for melanoma, but significantly enhances the susceptibility of malignant T cells to apoptosis. To determine if bryostatin might potentiate apoptosis in melanoma, we studied its effects on apoptotic pathways induced by death receptors or cytotoxic drugs. We conducted cytotoxicity experiments in WM35, a human radial growth phase melanoma cell line, and A375, a human metatstatic melanoma cell line. Treatment of these cells with bryostatin increased susceptibility to Fas-mediated apoptosis significantly. Bryostatin also potentiated the susceptibility of both melanoma cell lines to cisplatin-induced apoptosis. Interestingly, bryostatin showed a ''biphasic'' effect, inhibiting apoptosis at low concentrations, but potentiating susceptibility to apoptosis at higher concentrations, consistent with the biphasic effect of bryostatin on PKC. Our results suggest that bryostatin might be an effective adjuvant for immunotherapy or chemotherapy in melanoma, since it potentiated both Fas-mediated and cisplatin-induced apoptosis. Our initial studies on the mechanism indicate that the effect of bryostatin is complex, involving expression of death receptors as well as downstream antiapoptotic factors.

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Tumor Regression Induced by Intratumoral Injection of DNA Coding for Human Interleukin 12 into Melanoma Metastases in Gray Horses

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Introduction: Presently, preclinical studies investigating new therapeutic principles against melanoma are studied in mouse models, which are not optimal. Gray horses tend to spontaneously develop melanomas that subsequently metastasize in a pattern similar to human disease. Thus, they provide a highly relevant large animal model for preclinical studies testing new immunotherapy protocols. Methods: Seven gray horses with metastatic melanoma were included in the study. Tumor size was assessed before and regularly during the study using calipers, ultrasound measurements and endoscopic assessment. Established tumor metastases were injected with naked DNA coding for human interleukin 12 (IL-12) or control vector. Some lesions were left untreated. Biopsies and blood samples were taken and analyzed at specified time points during the study. Biological activity of human IL-12 in the horse was assessed by measuring induction of equine interferon gamma by real time RT-PCR and proliferation after stimulation with recombinant human IL-12. Results: Injection of human IL-12 encoding plasmid DNA induced significant regression in all 12 treated lesions in a total of 7 horses. Complete disappearance was observed in one treated lesion, with no recurrence after 8 months. No adverse events have been observed in any of the animals during and after treatment. Conclusion: The results demonstrate the effectiveness and safety of IL-12 encoding plasmid DNA therapy against established metastatic disease in a large animal model and serve as a basis for a clinical trial.

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Immunotherapy of Metastatic Melanoma with Tumorlysate-/or Peptide-Pulsed Monocyte Derived Mature Dendritic Cells Leads to Regression of Metastases

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Malignant melanoma is a highly aggressive tumor with increasing morbidity and mortality worldwide. To date there is no therapy for metastatic melanoma with predominantly curative effects. Based on the emerging concept of the central role of antigen presenting cells (APC) in the initiation of immune responses – especially the unique potency of dendritic cells (DC) in activating cytotoxic T-cells (CTL) – in the last years DC-based vaccines are under investigation. However, the best vaccination strategy still remains unclear. The elucidation of this question presents the central object of this study. We use tumorlysate-/or peptide-pulsed monocyte derived DC to induce a tumorspecific immune response by activation of tumorspecific CTL. In our phase II-trial 14 patients with progressing metastatic melanoma (state IV of UICC) have been investigated so far. The first 4 patients – vaccinated with immature DC – showed progress of disease within the induction period (< 5 weeks). Therefore the following 10 patients were treated with mature dendritic cells. We observed a clinical response in 5 cases: 3 patients showed a stable disease (SD) over a long period of time, 2 patients a partial remission (PR). 5 patients progressed in disease. One of these patients with PR responded with regression of extensive abdominal metastases, lymph node metastases and bone metastases, the other one with necrotic desintegration of abdominal lymph nodes. 4 of the patients with PR or SD were treated with autologous tumorlysate-pulsed DC, one with peptide-pulsed DC. To judge the performance of peptides/tumorlysate in vaccination strategies we investigated the functional stimulation of T-cells by ELISPOT analysis. First results show that a tumor-/peptide-specific CTL-response could be induced.

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Decreases in Serum Levels of S100 May Predict Response to Therapy in Melanoma Patients Treated with a Polyvalent Melanoma Vaccine

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Serum levels of S100 appear to be predictive of prognosis in patients with melanoma. In this study we explored whether changes in the level of this antigen could be used to evaluate. response to therapy. We measured serum levels of S100 at multiple time points during therapy with a polyvalent, shed antigen melanoma vaccine in 106 melanoma patients (58 with AJCC stage IIB/IIIA, and 48 with stage IIIB disease). S100 was measured immediately prior to treatment and following 2, 4, and 10 months of therapy using a double sandwich ELISA. We found that patients who were S100 positive at baseline and whose S100 levels decreased during vaccine treatment (n = 30) had a recurrence-free survival that was significantly longer than that of patients whose levels increased or remained stable during therapy (n = 11). By Kaplan-Meier analysis the mean recurrence-free survival was 12.5 ± 0.7 months in the first group vs. 9.2 ± 0.7 months in the second group. Patients who were S100 negative throughout the study (n = 65) had the longest recurrence-free survival, i.e. mean of 20.2 ± 0.8 months. These results suggest that serial assays of S100 in the circulation may provide an intermediate marker of the clinical effectiveness of melanoma vaccine therapy.

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Atypical Cells in Malignant Melanoma Re-excision Scars: An Immunohistochemical Study

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Background: Re-excision scars for melanoma may be hypercellular and contain atypical cells with prominent nucleoli mimicking residual disease. Immunohistochemical analyis with anti-S100 may be used to detect malignant melanoma cells within the scar. The specificity of anti-S100 is limited because it also labels dendritic and Schwann cells which appear to be increased in number and size in scars. No studies exist that address the question of the nature of these cells. Design: Ten skin re-excision scars for melanoma (MS) and five for non-melanoma cases (NMS) were studied by routine histology and immunohistochemical labelling for S100, gp100 (HMB45), and MART1. The presence of atypical cells, their morphology, and location were correlated with their immunolabelling pattern. Results: Numerous large, hyperchromatic, spindled to polygonal cells, with occasional prominent nucleoli were present mostly within the papillary dermis (10/10 MS, 5/5 NMS). They were often near or adjacent to blood vessels and surrounded by lymphocytes and were strongly labeled with anti-S100 (10/10 MS, 5/5 NMS). All were negative with HMB45 (0/10 MS, 0/5 NMS). In some cases, rare cells close to the epidermis were labeled with MART1 (4/10 MS, 4/5 NMS). In one MS case, MART1 was useful in detecting a focus of residual melanoma that was negative with HMB45. Conclusions: Atypical cells can be seen within re-excision scars and may be confused with residual malignant melanoma. Analysis of S100 expression alone may result in a false positive diagnosis of residual melanoma. Additional routine use of HMB45 and MART1 is, therefore, recommended in all re-excisions scars for melanoma. Further studies are required to delineate the origin of these cells.

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Evidence for an Association between Cutaneous Melanoma and Non-Hodgkin's Lymphoma

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For decades, both cutaneous melanoma (CM) and Non-Hodgkin's lymphoma (NHL) have increased in incidence dramatically. We looked for a possible relationship between these two cancers by examining the risk of NHL in CM survivors and the risk of CM in NHL survivors. To this end, we followed cohorts of CM and NHL patients registered through the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program between 1973 and 1996 and identified patients who developed CM after NHL and NHL after CM. To evaluate relative risk, we compared the observed number of cases to the expected number of cases. Between 1973 and 1996, a total of 54 803 CM patients and 62 597 NHL patients who met our inclusion criteria were identified through SEER. We found statistically significant elevated risks of NHL among CM survivors (SIR = 1.42, 95% CI = 1.23–1.63) and CM among NHL survivors (SIR = 1.75, 95% CI = 1.48–2.07). These results support an association between CM and NHL. Although detection bias and post therapy effects may contribute to this association, shared genetic or etiological factors, such as sunlight exposure, may also play a role.

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Risk of Death in Inherited Epidermolysis Bullosa (EB): Cumulative and Conditional Probabilities, Based on the Experience of the National EB Registry (NEBR), 1986–2000

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It is known that inherited EB may prove fatal. One subtype of junctional EB (JEB) was once named "EB letalis", to emphasize this risk. We have analyzed 14 years' data collected through 12/31/00 on the first 2650 enrollees in the NEBR to quantitate the risk of death in each major EB subtype. During this time, 7.5% of all NEBR enrollees had died, ranging from 0.9% of EB simplex (EBS) Weber-Cockayne to 53.5% of JEB-Herlitz (JEB-H) patients. Among EBS patients, the only significant subtype at risk of premature death (and only during the first year of life) was Dowling-Meara (EBS-DM), with a cumulative risk of 3.1% on or after age 1. With rare exceptions, death in JEB patients occurred primarily within the first 3 years of life. Non-Herlitz JEB (JEB-nH) and JEB-H patients had cumulative risks of 37.3% and 40.0%, 43.2% and 45.6%, and 43.8 and 48.5%, by ages 1, 2, and 3, respectively. The highest conditional probabilities for death in JEB-nH (36.7%) and JEB-H (40.0%) both occurred during the first year of life. There was no significant increased risk of death in dominant dystrophic EB (DDEB) patients. A bimodal distribution of deaths was seen in patients with Hallopeau-Siemens recessive dystrophic EB (RDEB-HS), with the highest interval at risk (conditional probability = 47.6%) between ages 35–40. Cumulative risks of 1.6%, 3.2%, 5.0%, and 7.9%, were seen by ages 1, 6, 10, and 15, and then rapidly increased to 20.1%, 35.4%, 44.4%, 63.0%, 80.6%, and 86.1%, by ages 20, 25, 30, 35, 40, and 45, respectively. A similar pattern occurred in RDEB-nHS, although the cumulative risks were much lower at every age interval, being 0.8%, 2.4%, 9.4%, 17.7%, and 29.9%, by ages 1, 15, 30, 45, and 55, respectively. These data suggest several conclusions: (1) Among EBS subtypes, EBS-DM is the only one with significant risk of premature death, and only during infancy; even then, the cumulative risk is only 3%; (2) Most JEB deaths occur within the first 3 years of life, with nearly identical cumulative risks in each of the two major JEB subtypes; (3) Death occurs much more often in RDEB-HS than RDEB-nHS, and in both, most deaths occur during adulthood; (4) Differences in when deaths occur suggest the likelihood of different etiologies, and further suggest differences, by EB subtype, in when patients require closest surveillance against possible risk factors.

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Inherited EB and the American Health Care System: Differences in EB Type in the Utilization of Inpatient and Outpatient Facilities and of Specialists, and Patient-Parental Satisfaction

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A cross-sectional study of approximately 400 randomly selected National EB Registry enrollees was undertaken to assess health care system utilization and patient perceptions of their care within the past 12 months. Adults and children had significantly different medical needs, as reflected in their relative use of specialists for the management of selected extracutaneous manifestations. 15% and 3.6% of all adult RDEB patients were seen by surgical oncologists and psychiatrists, compared to 0% and 13% of RDEB children, respectively. In contrast, children with JEB were more likely to be treated by gastroenterologists than were adults with JEB (18 vs. 8.3%). Overall, adults sought more frequent evaluation by dermatologists (JEB, 92 vs. 77%; RDEB, 75 vs. 63%) than did children. About 73% of all children with EB were treated by a pediatrician whereas less than half of all adults with EB required treatment by an internist or family practitioner. Most medical care for EB in the United States was provided as outpatients. 14% of adults and 29% of children with JEB, and 32% of adults and 25% of children with RDEB, required hospitalization. The average number of hospitalizations per year among those requiring inpatient care differed by age group and EB type (JEB:1.3 in children, 1.4 in adults; RDEB:1.7 in children, 2.0 in adults). Relative satisfaction in overall health care and specific physician attributes varied by EB type and age group. A recurrent complaint, especially by parents of more severely affected children, was that EB clinical research was proceeding too slowly.

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The Cost of Health Care for Inherited EB in the United States: Differences in Relative Coverage for Inpatient and Outpatient Services, and Overall Estimates of Yearly Expenses

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Approximately 400 randomly chosen enrollees in the NEBR, representative of all major EB types and subtypes, were studied in an effort to better assess the relative cost of medical care for each of these diseases within the United States. Patients (or their parents or guardians, if minors) were questioned in detail about their medical insurance coverage and overall costs during the 12 months preceding each interview. Considerable differences were noted across EB types as to the frequency with which patients experienced out-of-pocket costs for different aspects of their health care. Whereas over 80% of all EB children had all inpatient costs covered by some form of insurance or health care program, only about 60%, 35%, and 10–42% of all EB children had all costs related to outpatient clinic visits, private physician appointments, and prescription medications covered by some third party payer. Surprisingly, 15–20% and 42–51% of all JEB and RDEB patients had to pay out-of-pocket for over 76% of their costs for nutritional supplements and wound care supplies, respectively, despite the frequency with which each was required by these patients on at least a daily basis. Although considerable variations were noted within each major EB group, the annual average costs for medical care which was directly related to EB was estimated in children to be $430 for EBS, $846 for DDEB, $68,632 for JEB, and $11,747 for RDEB. This can be contrasted in adults with estimated costs of $753 for EBS, $542 for DDEB, $2,168 for JEB, and $29,365 for RDEB. These striking differences in overall costs of care for children versus adults with JEB and RDEB reflect differences in the relative frequencies of costly and clinically significant extracutaneous sequelae in childhood and adult life within these two major EB types.

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Activities of Daily Living (ADL) and Disease-Associated Pain in Children with Inherited Epidermolysis Bullosa (EB)

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Standardized questionnaires were used to assess the physical and emotional functionality of approximately 400 randomly chosen enrollees in the NEBR who represented all major EB types and subtypes of this disease which are seen within the United States. The level of independence of six ADLs (toileting; feeding; bathing; dressing; grooming; ambulation) were assessed, using conventional criteria. Whereas 90% of all EBS and DDEB children were totally independent for each function, excluding ambulation, the frequency of totally independent patients with JEB and RDEB ranged from only 42–73%. No DDEB children and only 2% of EBS patients were totally dependent by ADL, in comparison to 8–27% of JEB 2–27% of RDEB children. Totally independent physical ambulation was reported in only 31%, 31%, 67%, and 24% of EBS, JEB, DDEB, and RDEB children. Daily level of EB-related pain was assessed in children by their parents on a linear scale of 0 (no pain) to 10 (unbearable pain). Whereas 14–19% of all children with EBS, JEB, and DDEB were graded 5, 32% of all RDEB children reportedly suffered this much pain. Increased frequencies of pain 5 were most often noted in those with more clinically extensive or severe EB subtypes. These included JEB-Herlitz (20% vs. 14% in JEB-non-Herlitz) and RDEB-Hallopeau-Siemens (47% vs. 20% in all other RDEB subtypes). Conversely, only 5% of all RDEB children reportedly were pain-free, compared to 12–14% of those with EBS, JEB, and DDEB.

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Inherited Epidermolysis Bullosa (EB): Impact of an Affected Child on Parental Interpersonal Relationships, Marital Status, and the Decision to have Additional Children

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Standardized questionnaires were used to assess the impact of the presence of EB in one or more children on the personal relationships shared between their parents. In general, the presence of a severely affected child with EB had rather profound effects on many aspects of marriage. This ranged from complaints of lack of interest in participating in activities as couples (JEB, 45%; RDEB, 25%), lack of energy to invest in such pursuits (JEB, 82%; RDEB, 50%), limitations in opportunities for sharing nonintimate physical activities (reported in nearly 78% of EBS parents), and a significantly negative impact on parental sex life (among JEB parents, reported in 55%; in RDEB, in 39%). 10%, 64%, 25%, and 36% of parents of an affected child with EBS, JEB, DDEB, and RDEB, respectively, characterized their relationships as couples as revolving almost exclusively around the day-to-day care of their affected children. Only 46% and 19% of parents with a child with EBS or DDEB denied any global alteration in their marriage as a result of having one or more affected children. The severity of disease in an affected child clearly influenced parenteral decisions about having more children. 54% and 63% of parents of children with JEB and RDEB chose not to have additional children, compared to only 24–26% of parents with children having EBS or DDEB. This choice was most often pursued via tubal ligation; less often, alternative means of surgical sterilization were chosen. Divorce was common among EB parents (range, 17% in EBS to 31% in JEB) and, with the exception of parents of an EBS child, was usually directly attributed by one or more of the parents to the profound impact that this disease had exerted on their marriages.

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Inherited EB: Impact of an Affected Child on Parental Feelings of Guilt, Blame, Anxiety, Self-Image, and Depression, Religious Beliefs and Ability to Cope, and the Risk of Suicide in Affected Adults

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We have assessed the impact of a child with inherited EB on a variety of parental emotions or beliefs. Roughly 1/3–2/3 of all parents, regardless of the EB type present in their child or its genetic mode of transmission, admitted to feeling that their spouses were somehow to blame for this disease. 36%, 30%, 65%, and 38% of parents with a child with EBS, JEB, DDEB, and RDEB, respectively, blamed themselves, even in the absence of previous evidence of this disease within their families. A common complaint among parents was that ''nobody understood the personal burden of this disease'' (range, 44% in EBS to 80% in JEB parents), adding to their sense of isolation and despair. Religious beliefs in parents were reportedly changed, following the birth of an affected child, in only 5% and 14% of EBS and DDEB parents, as compared to 28–29% of parents of children with JEB and RDEB. In the majority of those who admitted to having experienced such a change, religious beliefs became more important by the presence of their affected child, as was their overall belief in God. Rare parents of children with JEB and RDEB, however, also concurrently admitted to blaming God for their childrens' disease. Fatigue, anxiety, altered self-image, and depression were common, especially among parents of more severely affected children. Approximately half of all JEB and RDEB parents complained of fatigue, and 85% and 80% of JEB and RDEB parents, respectively, compared the frequent fluctuations in their lives to those of ''someone on a roller coaster.'' On the other hand, learning to cope with and to manage their childrens' disease was described by over half of all EB parents as having made them better persons. Suicidal ideation (18–26%) and suicidal attempts (17–22%) were reported by a significant minority of adults affected with JEB and RDEB.

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Impact of an Affected Child with Epidermolysis Bullosa (EB) on the Functionality of the Family Unit and the Ways in which Parents are Regarded by Others within their Own Communities

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We have employed a detailed questionnaire on nearly 400 patients with inherited EB in an effort to assess the possible impact of their disease on their immediate families, relatives, and friends. In general, families having one or more children affected by JEB or RDEB were the most affected, regardless of the parameter which was assessed, when contrasted with families of children with DDEB and EBS. The financial burden of JEB and RDEB negatively impacted in many ways on affected families, to include the ability of the families to afford nonessential but still desirable purchases, to take family vacations or otherwise travel outside of their own communities, to eat outside of their homes, to afford to own their own houses (in 63% of JEB families; 24%, RDEB), to afford private education for unaffected children, or allow parents to pay for their own additional education. Over a quarter of all JEB families also reported having had to give up attending church as a result of the severity of their childrens' disease. About 25%, 46%, 19%, and 40% of parents of children with EBS, JEB, DDEB, and RDEB felt that that they were neglecting their other children as a result of the many burdens imposed on them by having an affected child with EB. A variety of antisocial behaviors were attributed to interactions with nonfamily members, including social ostracization (in 30–50%), false accusations of child abuse (38–70%), not being made to feel welcome by strangers (in 56–80% of non-EBS families), having their affected children excluded from play with other children, and concerns raised by others about EB being potentially contagious. Some parents also reported decreased interactions with previously close friends although no obvious changes were reported among relationships with relatives who were outside of the immediate family unit.

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Impact of Gene vs. Protein Replacement on Genomic Expression Patterns in Junctional Epidermolysis Bullosa

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Identification of the genes responsible for over 80 human genetic skin diseases provides a rational basis for development of new targeted molecular therapeutics. However, the relative efficacy of gene vs. protein-based therapies in achieving phenotypic correction is unclear. Similarly, the global impact of each approach on genomic expression patterns is unknown. While most molecular therapy studies have examined impacts on a limited number of parameters, different approaches may impact many biological systems within cells and tissue. In our efforts to develop an effective molecular therapy for junctional epidermolysis bullosa (JEB), we found that laminin 5 β3 gene transfer corrects the JEB skin phenotype *in vivo* including tissue ultrastructure and the localization of key proteins of the basement membrane zone. To model the relative effects of gene transfer vs. protein therapy for JEB, we cultured primary keratinocytes from six JEB patients following three different treatment regimens (1. nontreated, 2. gene therapy, 3. protein therapy) and compared cellular morphology, growth kinetics and gene expression profiles using cDNA microarrays encompassing 2068 known human genes; the latter were performed in triplicate for each patient and site matched normal control. While both protein and gene delivery restored JEB cell short-term growth kinetics to that of normal controls, β3 gene transfer resulted in cellular morphology and gene expression profiles more closely resembling normal than did β3 protein transfer. As anticipated, many of the genes whose expression was restored to the normal range following treatment were those encoding adhesion molecules and components of the hemidesmosomes. Although gene transfer normalized the expression of a higher percentage of genes than did β3 protein transfer, neither approach fully normalized expression of all of the genes examined. In addition, both approaches altered the expression of some genes, but protein transfer disrupted expression of a larger proportion of the genes examined. These observations indicate that gene and protein transfer exert different and unanticipated biological effects in primary JEB keratinocytes. These findings are likely to have broad implications for the development of new molecular therapies.

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A Prospective Nonrandomized Comparison of Efficacy and Safety of Intravenous Immunoglobulin to Conventional Therapy in Ocular Cicatricial Pemphigoid

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Ocular cicatricial pemphigoid (OCP) is a systemic autoimmune disease presenting as chronic cicatrizing conjunctivitis. Involvement of other mucous membranes or the skin can occur during the course of the disease. We performed a prospective nonrandomized comparison of efficacy and safety of intravenous immunoglobulin (IVIg) to conventional therapy in patients with OCP who initially presented with cicatricial pemphigoid involving skin and/or mucous membranes other than conjunctiva and whose disease progressed to involve the eye in spite of systemic immunosuppressive therapy. Eight patients in group A were treated with intravenous immunoglobulin after the diagnosis of OCP was established. The efficacy and safety of IVIg therapy were compared to a clinically similar group of eight patients recently treated with conventional immunosuppressive therapy (group B). The median time between initiation of therapy and clinical remission in group A and group B was 4 months and 7.5 months, respectively ($p < 0.01$). Recurrence of ocular inflammation was not observed in any patient in group A. At least one recurrence (median 1) was observed in five patients in group B (range 0–4, $p < 0.05$). No disease progression was recorded in group A. On the contrary, four eyes of three patients progressed to an advanced stage of OCP. Four patients in group A suffered from drug related side-effects in the course of IVIg therapy. The total number of side-effects recorded in eight patients in group B was 33 and was statistically significantly higher when compared to group A ($p < 0.001$). Intravenous immunoglobulin is more effective and safer in treatment of patients with OCP when compared to conventional immunosuppressive therapy.

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Successful Outcome with IVIg Therapy in Treatment Resistant Pemphigus Foliaceus

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Pemphigus foliaceus is a chronic autoimmune blistering skin disease which is commonly treated with oral corticosteroids and conventional immunosuppresive therapy. Pemphigus foliaceus in some patients can be refractory to systemic treatments and the resultant side-effects of prolonged immunosuppression can be potentially fatal Alternative therapies to control the disease by immunomodulation are needed. The purpose of this study is to report treatment outcomes in 11 patients with severe pemphigus foliaceus, refractory to Prednisone and immunosuppressive therapy. The development of serious and debilitating side-effects warranted treatment with intravenous immunoglobulin (IVIg). In 11 patients, selection criteria included a biopsy and immunopathologically proven pemphigus foliaceus, treatment resistance and/or side-effects to conventional therapy, and a minimum of 12 months of follow-up after cessation of IVIg therapy. IVIg was administered according to a defined protocol. Criteria for clinical response to IVIg included control of disease, duration of IVIg maintenance therapy, total duration of IVIg, total number of cycles, use of Prednisone and adjuvant immunosuppressive therapy, and the number of recurrences and relapses. The pre- and post IVIg data was statistically analyzed using the SAS UNIVARIATE and 2-sided Wilcoxon sign rank and sign tests. All the patients had an effective clinical response and remained in clinical remission after discontinuation of IVIg therapy. No serious side-effects from IVIg use were recorded. IVIg therapy is an appropriate, effective, and safe biological agent in inducing and maintaining prolonged clinical remissions in pemphigus foliaceus patients who fail to achieve this goal by conventional treatments. IVIg is effective as monotherapy but is needed for a prolonged period to achieve long-term remission. Use of IVIg therapy can prevent the development of serious and characteristic side-effects from Prednisone and adjuvant immunosuppressive agents.

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Intravenous Immunoglobulin Therapy Results in Long-Term Remission in Bullous Pemphigoid Patients Non-Responsive to Conventional Immunosuppressive Treatment

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Several patients with bullous pemphigoid do not respond to conventional immunosuppressive therapy consisting of oral Prednisone alone or in combination with another steroid sparing immunosuppressive drug. In spite of such therapies some patients do not go into a prolonged clinical remission and continue to have relapses and remissions. In 15 patients with recurrent bullous pemphigoid who were resistant to conventional therapy and had developed numerous side-effects to such therapy, treatment with intravenous immunoglobulin was administered. Details of clinical course, dose of Prednisone, immunosuppressive agents, their duration, side-effects, frequency of relapses and recurrence response to therapy, number of hospitalizations, total days hospitalized and quality of life were noted pre and post intravenous immunoglobulin therapy. The results were compared and statistically analyzed. Fifteen bullous pemphigoid patients that were resistant to treatment, upon receiving IVIg went into a prolonged sustained clinical remission. There was a statistically significant difference in the clinical course of disease pre and post IVIg. IVIg is a safe and effective agent in treating severe bullous pemphigoid patients resistant to conventional therapy. IVIg may also be useful in patients who have developed or have the risk of developing serious or potentially fatal side-effects to conventional immunosuppressive therapy. After clinical control is achieved IVIg therapy should be gradually withdrawn over a period of time and not abruptly discontinued.

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Treatment of Epidermolysis Bullosa Acquisita with the Humanized anti-Tac mAb Daclizumab

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Epidermolysis bullosa acquisita (EBA) is an autoimmune subepidermal blistering disease characterized by IgG antibasement membrane autoantibodies to collagen VII. Patients may present with blisters and erosions precipitated by trauma, dystrophic EBA, or widespread inflammatory lesions, inflammatory EBA. Since autoantibody formation in EBA patients is thought to be T cell-dependent, the degree of T cell activation in 3 patients (all male, ages 33–44 year) was assessed by quantitation of soluble Tac, a fragment of the a-subunit of the high affinity IL-2 receptor (CD25). Soluble Tac levels in all patients were elevated (highest random values: 2,430, 920, 560 [normal range 112–502 IU per ml]). Based on such findings, these patients were treated with a humanized murine monoclonal anti-Tac antibody, Daclizumab (1 mg per kg body weight, 6–12 IV treatments at 2–4 week intervals). All patients had a significant, rapid, and persistent decrease in lymphocyte CD25 expression (e.g. 38% to 0.7% 2 weeks after first treatment). Though a slight decrease in lymphocyte expression of 7G7, an IL-2 receptor epitope not bound by Daclizumab, was noted, stable levels of CD3 cells and *in vitro* saturation studies indicated that Daclizumab effectively bound CD25 and did not promote clearance of such cells from peripheral blood. There were no complications and no patient developed antibodies against Daclizumab. While no apparent benefit was seen in patients with dermolytic disease, the patient with inflammatory EBA had a favorable response. This patient stopped prednisone and significantly reduced dapsone dosage while on Daclizumab. Furthermore, his disease flared when treatment was stopped, and resumption of Daclizumab again effected improvement within 2 weeks. Daclizumab therapy is safe and well tolerated in EBA patients. It may be effective as a corticosteroid sparing agent in patients with inflammatory EBA.

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Anti-CD20 Chimeric Monoclonal Antibody (Rituximab) for the Treatment of Recalcitrant, Life-Threatening Pemphigus Vulgaris: Implications for its Use in Other Autoimmune Antibody Mediated Diseases

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The development of chimeric antibodies which target constituent cellular antigens is expected to radically alter our approach to neoplastic and immunological disorders. Rituximab (Rituxan), a chimeric monoclonal antibody directed against CD20 of B cells has been used in patients with B-cell Non-Hodgkin's lymphoma with good effects. Over the past year, there have been anecdotal reports of the use of rituximab in autoimmune disorders, specifically, a case of autoimmune thrombocytopenia and a small series (10 patients) with rheumatoid arthritis. In both settings, rituximab had a dramatic effect on the targeted condition. In this report, we describe the use rituximab in a patient with refractory pemphigus vulgaris (PV). The case concerns a 30-year-old woman who failed to improve with conventional therapies for PV including: systemic steroids (continuous prednisone and pulsed methylprednisolone), azathioprine, continuous and pulsed cyclophosphamide, plasmapheresis, intravenous gamma globulin, and mycophenolate mofetil. Due to recurrent bouts of life-threatening septicemia with multiple-drug resistant microbes, we opted to treat her PV with weekly infusions of rituximab (total of 6). After the first infusion, there was a complete obliteration of her B cells, which has remained nondetectable 4 months post-treatment. Similarly, her PV titers dropped dramatically after the first two infusions. Despite elevated PV titers (range 1:80–1:640), her skin has completely re-epithelialized. Knocking-out plasma cell precursors (i.e. Pro-B cells to mature B cells) presumably temporarily, in patients with auto-antibody mediated disorders such PV may be lifesaving in those individuals recalcitrant to conventional therapies.

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Comparison Between IVIG and Conventional Immunosuppressive Therapy Regimens in Patients with Severe Oral Pemphigoid: Effects on Disease Progression in Patients Nonresponsive to Dapsone

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Mucous membrane pemphigoid initially limited to oral cavity may so remain or progress to involve other mucosae. In patients with severe progressive disease, treatment with dapsone is recommended. We treated 20 patients in whom dapsone could not be used. Eight patients received intravenous immunoglobulin therapy (IVIg) and 12 received oral prednisone with an immunosuppressive agent as conventional therapy. After initiation of therapy, patients in both groups were followed for a mean of 60 months. Clinical outcome was measured by duration of treatment, side-effects, rate of recurrence, relapse and remission, progression of disease to involve extraoral mucosal sites, and quality of life. Statistical analysis demonstrated that the two groups were similar with respect to age, gender, duration of disease prior to treatment, dose and duration of dapsone treatment, and total length of follow-up. The IVIg treatment group compared to the conventional treatment group showed statistically significant less duration of treatment, fewer relapses, more remissions, fewer side-effects from treatment, less extra oral disease progression, and a better subjective quality of life. IVIg is a safe and effective modality to treat mucous membrane pemphigoid. It appears be a good option for patients who cannot be treated with dapsone and in whom conventional therapy is contraindicated or results in the development of serious side-effects. In patients with progressive mucous membrane pemphigoid, IVIg may arrest disease progression.

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Difference between Prodromal Bullous Pemphigoid and Fulminant Bullous Pemphigoid

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We have undertaken a retrospective study of bullous pemphigoid. We looked at a group of 53 patients, 22 treated at an academic medical center and 31 treated by community dermatologists. We found that more patients were diagnosed with bullous pemphigoid in its prodromal stage when the patients were treated in an academic setting compared to a community setting (78% vs. 39%). We found that patients were more often treated with systemic steroids and immunosuppressive agents when they were treated in an academic center compared to a community setting (91% vs. 52% treated with systemic steroids, 50% vs. 16% treated with immunosuppressive agents). We also detected a trend toward younger age at diagnosis of prodromal bullous pemphigoid compared to fulminant bullous pemphigoid (average age 72 vs. 78). Patients with prodromal bullous pemphigoid could easily be controlled with lower dose of systemic steroids (40–60 mg prednisone q.o.d. or high potent topical steroid. Prodromal bullous pemphigoid ran shorter clinical course than that of fulminant bullous pemphigoid (1 years vs. 2+ years). In conclusion, bullous pemphigoid can be diagnosed at an earlier stage as prodromal bullous pemphigoid, especially in an academic setting. Prodromal bullous pemphigoid runs a milder and shorter clinical course.

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Novel Animal Model for Testing Anti-Acantholytic Treatments of Pemphigus

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High doses of corticosteroids is the life-saving therapy of autoimmune pemphigus but the mechanism of action is not completely understood. Methylprednisolone blocks pemphigus vulgaris (PV) antibody-induced acantholysis in skin organ cultures, suggesting direct antiacantholytic effect on keratinocytes (KC). However, dexamethasone does not block PVIgG-induced acantholysis in 1–2 d old Balb/c mice, suggesting a role for immunosuppression. We found that 3–5 d old Balb/c mice may respond to antiacantholytic treatments with corticosteroids or cholinomimetics, but untreated positive controls do not always produce PV lesions, because rapidly developing hair follicles may reinforce their epidermal integrity. Therefore, we sought to develop a more reliable animal model, and tested athymic nude mice. The neonates injected with 10 mg per g per day of PVIgG at different ages developed clinical and histologic signs of PV. The 1–2 d old pups died despite any antiacantholytic treatments, whereas the 5–7 d old pups responded to treatments and survived subsequent injections of PVIgG given together with a test drug. Therefore, we selected 5–7 d old athymic nude mice weighting ~2 g as a model for testing antiacantholytic drugs. The extent of acantholysis was assayed by measuring the length of intraepidermal split, at least 4 basal cells long, in at least 5 different microscopic fields (×100), and expressing results as percent of the total length of epidermis in the field, taken as 100%. All mice treated with methylprednisolone, 15 μg per g per d, survived at least 3 injections of PVIgG. Microscopically, the extend of acantholysis decreased from 77.5 ± 2.3% in nontreated control to 22.5 ± 4.3% ($p < 0.05$). Carbachol, 40 ng per g per day, decreased the extent of acantholysis to 40.8 ± 5.1% ($p < 0.05$). Neither drug prevented PVIgG from binding to mouse KC, as determined by semiquantitative immunofluorescence, thus illustrating direct antiacantholytic effects of both drugs on KC. Methylprednisolone could protect KC from acantholysis by increasing their expression of the PV antigen pemphaxin. To test this hypothesis, we compared relative amounts of pemphaxin in the epidermis of intact mice injected with 15 μg per g per d of methylprednisolone and found an increase from 31.5 ± 4 to 59.3 ± 7.0 ($p < 0.05$). Thus, our novel animal model of PV allows accurate testing of the efficacy of antiacantholytic drugs. The direct antiacantholytic action of corticosteroids on KC can be mediated, at least in part, by overexpression of pemphaxin.

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Systemic Isotretinoin and Recessive Dystrophic Epidermolysis Bullosa (RDEB): Results of a Phase 1 Clinical Trial

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RDEB patients are at high risk for the development of squamous cell carcinomas (SCCs); most will die of metastatic SCC within five years of their diagnosis. This argues strongly for the need for some form of effective chemoprevention. Intriguing data in many other diseases suggest that systemic isotretinoin may be effective as a chemopreventive agent against a variety of tumors. An 8-month, nonblinded, Phase 1 trial was undertaken on 20 patients with RDEB, aged 15 or older, to determine whether patients with this disease would be able to tolerate this drug at a targeted maintenance dosage of 0.5 mg per kg per day. Patients ranged in age from 15 to 67 years; 15 and 5 had generalized forms of non-Hallopeau-Siemens (RDEB-nHS) and Hallopeau-Siemens (RDEB-HS) disease, respectively. Drug-induced hypertriglyceridemia occurred in one. Four of the remaining 19 were unable to achieve maintenance dosage. Common complaints included increased dryness of the skin (65%) or oral cavity (20%), skin fragility (45%), pruritus (25%), and epistaxis (20%). Most of these, however, were relatively mild or infrequent. One patient developed excessive granulation tissue which resolved at a lower dosage. No patients developed new tumors while on therapy, although such an effect was not expected to occur within this limited time period. RDEB subtype did not appear to influence the relative frequency of subjects developing increased dryness of the skin or mouth, or of epistaxis. Only 3 of 19 subjects now eligible for Phase 2 treatment wishes not to receive further treatment, attesting to the reasonable level of tolerance to systemic isotretinoin at this dosage, as well as patient interest and motivation. Intriguingly, 55% of all subjects (80%, RDEB-HS; 47.7%, RDEB-nHS) reported some clinical lessening in blister formation while on systemic isotretinoin.

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Osteopenia and Osteoporosis in Recessive Dystrophic Epidermolysis Bullosa (RDEB): Results of DEXA scans in 42 Consecutively Studied Patients within the United Kingdom and United States

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It is well established that malnutrition occurs in patients with the more generalized forms of recessive dystrophic EB (RDEB), especially in those with the Hallopeau-Siemens (RDEB-HS) subtype. In other debilitating conditions, chronic immobility may contribute to development of osteopenia or osteoporosis. Data are lacking as to whether patients with RDEB are at risk of developing decreased bone mass. We have therefore performed DEXA scans on 42 consecutive patients (male, 23; female, 19) with RDEB, aged 15–66, who were evaluated at the two largest referral sites for EB patients within the United Kingdom and United States (the latter affiliated with the National EB Registry). 25, 11, 4, and 2 patients had HS, generalized mitis, inversa, and localized forms of RDEB. 43% and 41% had evidence of osteoporosis or osteopenia, respectively. Abnormal findings were most commonly seen in RDEB-HS, with 60% and 32% having osteoporosis or osteopenia. Surprisingly, only 27% of mitis patients had normal DEXA scans; 18% met criteria for osteoporosis and and the remaining 55% had osteopenia. A similar distribution was seen among inversa patients. Gender had no apparent effect on the distribution of patients having osteoporosis, osteopenia, or normal scans. When stratified by age, abnormal DEXA scans were most commonly seen in younger aged patients. Only 8% of those under 21 had normal scans and 75% showed evidence of osteoporosis. Taken collectively, these findings suggest that osteopenia and osteoporosis are common findings among all of the major RDEB subtypes, even in those subtypes not usually associated with clinically profound growth retardation.

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Graftskin Therapy in Epidermolysis Bullosa

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Graftskin is a bi-layered human skin construct which is derived from foreskin keratinocytes and fibroblasts. It is approved for the treatment of venous leg and diabetic foot ulcers. As it contains none of the keratin gene mutations of epidermolysis bullosa (EB), we have used graftskin to treat a series of children with EB including: 2 Dowling-Meara-type EBS (#sites = 30), 1 Weber-Cockayne EBS (#sites = 6); 1 Herlitz-type JEB (#sites = 2) and 2 RDEB (#sites = 39). All active lesions were debrided prior to application of fenestrated graftskin. Wounds were dressed with nonstick gauze, a hydrofoam dressing and a compression wrap. Graft take and persistence was assessed clinically @ weeks 1 and 4 and by EM and analysis for donor DNA @ weeks 3, 4, 6, 12, 20. All sites treated in EBS and RDEB showed 90–100% healing by 1 week post grafting, with many of the sites appearings as normal skin by 10–14 days. Two RDEB cases had mitten deformity release procedures with finger web space graftskin reconstruction, resulting in 50–75% increase in range of motion @ 6 weeks. Recurrences were common, but were limited to only the most highly trauma prone areas (plantar feet, knees and elbows) and responded well to repeat grafting. The children's parent's all reported diminished pain, less bleeding and improved ambulation after graftskin treatment. EM showed normalization of tonofilament clumping at 6 weeks in 1 child with DM-EBS. PCR analysis using primers for a donor-specific HLA-Dβ1 allele revealed persistence of donor specific DNA @ 3 and 12 weeks in 2 cases, no donor DNA in 1 case @ 4 and 20 weeks and 2 cases that shared the same allele as the donor. DNA fingerprint analysis failed to detect any donor specific sequences in 7/7 samples. The encouraging clinical results reported here support that graftskin is doing more than simply functioning as a bandage or a source of growth factors to stimulate autologous closure of the wounds in EB. Although most of the molecular analyses failed to document long-term persistence of donor DNA, ultrastructural and clinical features of treated sites do suggest a long-term, dynamic effect of graftskin in EB patients. Future studies will require longterm follow-up of treated sites and more specific molecular probes to determine what the precise mechanism of action may be. Even if long-term/permanent engraftment is not realized in these children, the improved quality of life and the achievement of development milestones makes this an exciting step forward in the care of the EB patient.

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The *In Vitro* and *In Vivo* Effects of Slow-Release Antiseptics

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Antiseptics have become more important therapeutic agents in view of increasing bacterial resistance to antibiotics. Moreover, the slow-release mode of new antiseptics preparations is allowing their clinical use with minimal or no toxicity. In these studies, we used a prototypic slow-release antiseptic preparation, cadexomer iodine (CI), which carries iodine [0.9% (w/w)] immobilized in bead molecules of dextrin and epichlorhydrin, and which is highly effective in the management of exudative wounds. Our hypothesis has been that, within a certain concentration range, CI is nontoxic to cells and tissues and can also trap microorganisms within its cadexomer component. We first exposed cultured neonatal foreskin fibroblasts to increasing concentrations of CI [(0.45%-1.4% (w/v)] for 24 h. The use of up to 0.45% CI was not associated with any decrease in cell viability, as determined by trypan blue exclusion. In an attempt to mimic more closely the *in vivo* situation, cultured fibroblasts were exposed daily to a fresh preparation of CI (0.45%) for up to 120 h. We found no decrease in cell viability using this experimental protocol of more intense exposure of fibroblasts to CI ($p > 0.05$). To determine the effects of wound fluid on CI beads, swabs of CI, after being placed for 24 h in an exudative ulcer, were formalin fixed and gram stained. Microscopic analysis showed bacteria trapped within the beads. Histological evaluation of biopsies taken from ulcers treated with CI showed no morphological evidence of cell toxicity. In summary, fibroblast cell toxicity with CI is dose dependent *in vitro*. However, there are optimal CI concentrations, which even with prolonged incubation are nontoxic to dermal fibroblasts. For the first time, we also provide evidence that bacteria can be trapped in the cadexomer beads, probably independently of the iodine component. These data with this prototypic agent add to the emerging view that slow-release antiseptics can be safely used, and provide a framework for testing additional preparations.

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Tissue-Engineered Derived Growth Factors as a Topical Treatment for Rejuvenation of Photodamaged Skin

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Growth factor containing conditioned medium from the manufacture of three-dimensional tissue-engineered dermal constructs using human dermal fibroblasts was tested in *in vitro* studies and in safety and efficacy studies to assess their ability to promote skin cell growth and collagen deposition. Clinical safety studies with daily application of concentrated medium on sun-damaged forearm showed no irritancy or adverse reactions. Dermapathological evaluation and computerized histomorphic analysis of biopsies of treated areas showed a 50–80% increase in Grenz zone collagen in 2 of the 3 formulations designed to optimize growth factor penetration. Computerized measurement of tissue density and texture showed a 42–53% increase in newly deposited fine matrix fibers. These results are consistent with *in vitro* results which showed a statistically significant increase in both fibroblast and keratinocyte proliferation, collagen deposition, and antioxidant activity. These studies indicate that medium from tissue-engineered skin products may provide a unique benefit as a topical agent which can result in skin remodeling and regeneration.

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Wound Healing in Leg Ulcers Treated by Combined Skin Allograft and Autograft

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Combined transplant of skin allograft and autograft is a useful surgical method for chronic nonhealing leg ulcers. The role that homologous dermis plays in guiding wound healing is unclear under these conditions. The aim of the present study was to evaluate expression of certain cytokines involved in the healing process and certain adhesion molecules expressed by immunocompetent cells that may be involved in reject. Twelve patients with chronic nonhealing leg ulcers were treated by cryopreserved de-epidermized dermal (DED) allograft followed by autologous skin mesh grafts. The allografts were obtained from living donors undergoing plastic surgery. They were de-epithelized enzymatically, incubated with 15% glycerol for 2 h at 4°C, γ-irradiated with 60 Gy and then frozen at −80°C until use. Autologous mesh grafts were placed over the wound bed, 7–10 days after grafting with DED. Biopsy samples of transplanted skin were obtained 7, 14 and 28 days after grafting. Sections were examined by classical histology, transmission electron microscopy and immunohistochemistry (CD3, CD4, CD8, CD68, IFN-γ, TNF-α, endothelin-1, EGF, MIB-1). DED did not seem to give rise to any reject reaction that could prejudice taking of the autologous mesh. When it was possible to compare healing by both methods in the same subject (allograft with DED + autograft vs. spontaneous healing), healing guided by DED was found to produce a less sclerotic dermis, confirming that DED truly guides regeneration of physiological connective tissue.

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Apligraf® Counteracts the Growth Inhibitory Activity Present in Chronic Wound Fluid

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Chronic Wound Fluid (CWF) collected from venous ulcers was shown to inhibit growth of dermal fibroblasts, contributing to the impaired healing. To determine whether Apligraf® could counteract this growth inhibitory activity, thus promoting the healing process, effects of Apligraf® on the growth of dermal fibroblasts in presence of CWF was examined. Dermal fibroblasts were cultured from a biopsy taken at the edge of the venous ulcer. Paired cultured were plated onto transwell at 3000 cells per well and sterile cotton filter papers with two 8 mm punch biopsies of Apligraf®, epidermal side exposed to the air, were placed. Then CWF or Bovine Serum Albumin (BSA) as control was added at the concentration of 500 μg per well. Cells were allowed to grow for two weeks with two feedings per week containing fresh CWF or BSA. At the end of two weeks, the total cell number in each transwell was determined using Coulter Particle Counter. CWF inhibited the growth of fibroblasts as expected (250 000 vs. 160 000 cell per well). In presence of Apligraf®, CWF-induced growth inhibition was counteracted and cells grew at a normal rate (160 000 vs. 300 000 cells per well). When CWF collected from five different venous ulcers was tested, Apligraf® increased the growth of fibroblasts by 1.7–2.0 folds above that of the growth in the absence of Apligraf® in all CWF tested. In cells treated with only BSA, Apligraf® also increased the growth of the fibroblasts by 1.5–2.0 folds. Moreover, Apligraf® counteracted CWF with a wide range of growth inhibitory activity (from 32 to 86% inhibitions). Therefore, these results demonstrate that Apligraf® enhances basal growth of dermal fibroblasts cultured from chronic venous ulcers. In addition, Apligraf® can further stimulate healing of chronic venous ulcers in part by counteracting the growth inhibitory activity present in CWF.

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The Role of Dermal Skin Substitutes in the Management of "Hard-To-Heal" Unusual Wounds

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Dermal skin substitutes have been shown to be effective in the treatment of both full-thickness, nonhealing diabetic neurotrophic foot ulcers and "hard to heal" venous leg ulcers. Case studies have suggested that these skin substitutes may also have a role to play in managing wounds in patients with recessive, dystrophic epidermolysis bullosa (RDEB). Dermal skin substitutes were therefore applied to four patients with this condition. Areas of persistent ulceration, at multiple body sites, were treated with skin substitutes on successive occasions. Over an eight-week period, between 50 and 100% graft take was noted in all patients. Ulcers involving the pretibial area of the lower leg with exposed bone in the base of the ulcer can result from osteomyelitis or trauma. These ulcers often represent a therapeutic challenge as they fail to heal by secondary intention and there is no vascular supply within the base to allow skin grafting. Two patients with such ulcers were treated. Burr holes were drilled into the bone base and a dermal skin substitute was applied, on successive occasions, over the ulcer and secured to the surrounding skin. Islands of granulation tissue subsequently appeared around the burr hole openings. These merged to become confluent with the wound margins, resulting in a 30–50% reduction in wound size. Dermal skin substitutes with living fibroblasts appear to stimulate wound healing in patients with RDEB and exposed bone in the ulcer base. In addition to control of bacterial burden, overlap technique and adequate anchoring of the skin substitute appear to be important factors in facilitating healing.

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Keratinocyte Growth Factor-2 (KGF-2) Reverses Delayed Healing in Patients with Venous Ulcers and High Bacterial Burden at Presentation

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The effects of bacterial burden and healing rates on wound closure were evaluated in a 15 center, randomized, double-blind, 94 patients study of the safety and efficacy of topically applied KGF-2 in patients with venous ulcers (VU). Patients were eligible for enrollment in this 12-week study if they had a VU of 3–30 cm^2 in size and 3–36 months in duration. Bacterial burden was evaluated by tissue biopsy. Patients with a bacterial colony count 10^6 per gram of tissue were excluded but could be enrolled if after initial wound management had a subsequent colony count of $< 10^6$. The rate of wound healing was prospectively calculated based on the modified Gilman equation. We found that complete wound closure occurred in 29 (40%) of 72 patients with an initial colony count $< 10^6$ compared to 2 (9%) of 22 patients with a colony count 10^6 ($p < 0.01$). This effect was independent of wound size and duration. For the latter 22 patients, at least 75% healing was achieved by 62% (8/13) and 11% (1/9) of KGF-2 and placebo treated patients, respectively ($p = 0.03$). Thus, presentation with a high bacterial count was associated with a failure to heal even when measures were taken to reduce counts. However, KGF-2 appeared to remedy this situation. The rate of healing in the first 4 weeks of treatment was predictive of complete wound closure. An increased rate of healing was observed in the KGF-2 treated group. In conclusion, bacterial burden at presentation and the rate of healing during the first 4 weeks of treatment were predictors of complete wound closure. The data indicate that KGF-2 may reverse the association between increased bacterial burden at presentation and delayed wound healing.

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Mean Healing Rates at 4 Weeks can Predict Complete Wound Closure of Diabetic Foot Ulcers

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In a prospective, multicenter trial, 96 control patients with diabetic foot ulcers were treated with standard therapy consisting of aggressive debridement, moist wound dressings, and offloading with pressure relieving footwear, wheelchairs and/or crutches for 12 weeks. The objective of the healing rate analysis was to determine if the mean healing rate at 4 weeks was predictive of complete wound closure. Wound tracings were obtained at each visit, and computerized planimetry was used to evaluate ulcer area. Healing rates (HR) in cm per week were caluculated using an established formula ($\Delta A/\Delta p$, A = Area, p = perimeter). At study completion, we determined the mean HR at 4 weeks for all healers (n = 36) and nonhealers (n = 60). Wounds that completely healed during the study had increased mean HRs of 0.11 cm per week at 4 weeks (95% CI between 0.09 cm per week and 0.13 cm per week). Wounds that did not heal showed decreased mean HRs of 0.05 cm per week (95% CI between 0.04 cm per week and 0.07 cm per week). The mean HRs of healers compared to nonhealers were statistically significant at 4 weeks ($p < 0.0001$). A sensitivity and specifically analysis were performed using a target HR0.075 cm per week to predict complete wound closure. 28 of 36 patients were correctly identified as nonhealers with a HR < 0.075 cm per week. We conclude that a target HR0.075 cm per week is 78% sensitive and 80% specific in predicting complete wound closure in diabetic foot ulcer patients.

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Quantitative Assessment of Psoriasis Patients' Preference for Foam Vehicle

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Patients' acceptance of the vehicles used in topical therapy contributes to adherance to the treatment plan and subsequent outcomes. Betamethasone valerate in foam vehicle (Luxiq, Connetics Corp) has greater efficacy for scalp psoriasis than other betamethasone valerate preparations. The foam preparation is non-greasy, easily applied and residue free; thus, it is more appealing to patients than cream or ointment preparations. We sought to quantify patients' preference for this vehicle. Focus group sessions were held with patients with psoriasis to determine patients' perceptions of the advantages and disadvantages of different topical psoriasis therapies. This information was used to derive a "treatment adversity measure" to assess different topical therapies. 20 patients with psoriasis sampled different topical psoriasis medications and completed the "treatment adversity measure" for each. The foam preparation was much preferred over cream, ointment, gel and emollient preparations. This foam product provides an opportunity to achieve improved compliance with topical corticosteroid treatment.

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HLA-Cw*0602 but not Promoter Polymorphism of TNF-α is Associated with Development of Psoriasis in Japanese Patients

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The population survey with human leukocyte antigen (HLA) has provided evidence that susceptibility to psoriasis is linked to HLA with racial differences. Tumor necrosis factor (TNF)-α gene is also mapped within the HLA region and its product has been reported as one of the most important cytokines in the pathogenesis of psoriasis. In the present study the association of HLA genotypes and promoter polymorphism of the TNF-α with susceptibility for development of psoriasis vulgaris (PsV) was investigated. A hundred Japanese patients (76 males and 24 females) suffering from PsV were studied (the median age was 56 y, ranged from 15 to 90). Among them 32 patients were before age 40 (type I) and 68 were 40 or over age 40 (type II). Cw*0602 was significantly increased in the type I patients as compared with type II patients (25.0% vs. 7.4% $p = 0.0144$). On the other hand, there were no significant differences in the promoter polymorphism at −238 and −308 of TNF-α gene. These results suggest that Cw*0602 also is the main association of psoriasis in Japanese patients as peviously reported in Caucasians. However, TNF-α may vary across racial backgrounds.

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Using an Administrative Database to Assess the Risk of Malignancy Associated with Psoriasis

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The goal of this study was to measure the incidence of cancer in patients with psoriasis, stratified by the severity of their disease as determined by the use of systemic pharmaceuticals. This was a cohort study that utilized a database of administrative claims records from Medicaid programs in three US states. All individuals in the claims database were included in the study if they qualified for one of five groups: severe psoriasis, as defined by systemic medication use; less severe psoriasis; severe eczema; history of organ transplant; and hypertension. For each individual it was determined whether they had a diagnosis of cancer more than 6 months after they qualified for group classification. Individuals with severe psoriasis were more likely than those with hypertension to develop a malignancy (risk ratio: 1.78; 95% CI: 1.32. 2.40). The risk of malignancy in the severe psoriasis group approached that seen in organ transplant patients (risk ratio 2.12; 95% CI: 1.80, 2.50). Most of these cancers were due to nonmelanoma skin cancers and lymphoproliferative malignancies. Those with less severe psoriasis were only slightly more likely to develop a new malignancy than those with hypertension (1.13; CI: 1.03, 1.25). In conclusion, patients with psoriasis were at an increased risk of developing a malignancy as compared to patients with hypertension. This increased risk was greatest for those with severe disease (i.e. psoriatics using systemic agents) and was minimal, if at all, for those with less severe disease, compared to those in the hypertension group. Many of these malignancies were lymphoproliferative cancers and nonmelanoma skin cancers.

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Infliximab Clears Psoriasis in 80% of Patients With Moderate to Severe Disease

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TNFα recruits lymphocytes, stimulates intracellular adhesion molecule expression, stimulates neutrophil and monocyte chemotaxis, and induces other inflammatory mediators, such as Il-1, Il-6, and Il-8 in psoriatic plaques. Extracts of psoriatic lesional skin have significantly greater concentrations of TNFα compared to noninvolved skin. Infliximab is a chimeric monoclonal IgG antibody that binds to both soluble and transmembrane TNFα thereby inhibiting its activity. In this investigator-initiated, single site, randomized, double-blind, placebo-controlled clinical trial, 33 patients with moderate to severe plaque psoriasis were randomized to receive infliximab infusions at 5.0 mg per kg, 10.0 mg per kg or placebo in a 1:1:1 fashion. Patients were not allowed the use of any other concomitant therapy for psoriasis. Administration of either intravenous infliximab or placebo occurred at weeks 0, 2 and 6. Patients were assessed for response at week 10, which was defined as a ''good'', ''excellent'' or ''clear'' on the Physician's Global Assessment. A total of three patients withdrew during the course of the study, one from each group. The proportion of responders were 9/11 (81.8%) and 10/11 (90.9%) in the infliximab 5.0 mg per kg and 10.0 mg per kg groups, respectively, vs. only 2/11 (18.2%) patients in the placebo group ($p < 0.05$, Fisher's Exact Test for each infliximab vs. placebo comparison). Nine out of 11 (81.8%) and 8/11 (72.7%) patients had 75% improvement in PASI in 5.0 mg per kg and 10.0 mg per kg dosing groups, respectively, compared to only 2/11 (18.2%) in the placebo group ($p < 0.05$, Fisher's Exact Test for each infliximab vs. placebo comparison). The median percent improvement in PASI was 93% and 95% in the infliximab 5.0 mg per kg group and 10.0 mg per kg group, respectively, vs. 11% for the placebo group. The median time to response was 4.0 weeks for both infliximab groups. There were no serious adverse events and the drug was well tolerated. In conclusion, infliximab is the only biologic agent to date to clear psoriasis in a high proportion of patients in a manner comparable to cyclosporine.

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Circulating CD4+ and CD8+ Memory-Effector (CD45RO+) T Cells: Pharmacodynamic Markers During Alefacept Therapy

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Alefacept (human LFA-3/IgG1 fusion protein, LFA3TIP, currently being developed under the trade name AMEVIVE(tm)) binds CD2 and specifically and reversibly reduces memory-effector T cells (CD4+CD45RO+, CD8+CD45RO+). We tested if CD45RO+ subset reduction correlated with clinical improvement in 229 chronic plaque psoriasis patients (pts) who were randomized to placebo or alefacept (0.025, 0.075 or 0.15 mg per kg IV) weekly for 12 week. Circulating T-cell subset levels and changes in PASI were monitored weekly during therapy and 4 times postdosing over 12 week. Effects on CD45RO+ subsets were quantified by ''effect area under curve'' (EAUC). CD45RO + EAUC and percentage change in PASI for each patient were correlated. An important correlation was found for CD8+CD45RO+ ($r = -0.3361$, $p = 0.001$) and CD4+CD45RO+ cells ($r = -0.3154$, $p = 0.001$). Pts in the 0.075-mg per kg group were put in 3 groups based on T-cell changes in the first 4 week of dosing and response (= 75% PASI reduction 12 week after last dose) was compared. Pts with the greatest reductions in CD8+CD45RO+ (= 62% reduction) had a 60% response, compared to 27% and 7% in the other groups. Pts with the greatest reductions in CD4+CD45RO+ (= 45% reduction) had a 53% response, compared to 21% and 20% in the other groups. 62 pts were retreated (within 1 years) with alefacept 7.5 mg IV weekly for 12 week. Results showed similar reversible effects on CD45RO+ subsets and significant correlations between EAUC and PASI reduction for CD4+CD45RO+ ($r = -0.4629$, $p = 0.001$) and CD8+CD45RO+ ($r = -0.4576$, $p = 0.001$). These data provide novel insights for circulating CD45RO+ T cells as a predictor of response in psoriasis and suggest that peripheral T cells contain the pathogenic mediators of disease. This is the first report of a relationship between a specific T-cell subset reduction and clinical outcome in an autoimmune disease. Alefacept-mediated reduction of CD45RO+ cells appears promising for chronic plaque psoriasis.

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Gene Profiling of Blood Cells from Psoriatic Patients after Oral Treatment with SDZ ASM 981

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SDZ ASM 981 is a selective inhibitor of inflammatory cytokine release, specifically developed for the treatment of inflammatory skin diseases. Topical SDZ ASM 981 is efficacious in atopic dermatitis and allergic contact dermatitis without the side-effects of topical corticosteroids. In the initial four week study, oral SDZ ASM 981 was shown to be effective and safe in patients with moderate to severe plaque psoriasis. In the context of this study, blood samples were collected from 9 patients before and after 13 or 14 days of treatment (7 patients treated with 30 mg b.i.d. SDZ ASM 981 and 2 placebo-treated patients). Samples were subjected to gene expression analysis using gene chips allowing the survey of 7129 genes. A common genomic profile of SDZ ASM 981 (about 100 genes) was identified. At the mRNA expression level, the compound was shown to strongly down-regulate the expression of genes belonging to the macrolactam target pathway, e.g. macrophilin-12, calmodulin, cellular activation and proliferation (histone 2, histone 3.3, cyclin D2), inflammatory mediators (leukotriene A4 hydrolase, prostaglandin endoperoxide synthase), chemotaxis and cellular migration (LFA-1, P-Selectin ligand, L-Selectin, RANTES) as well as of HLA class II (Ii invariant chain, CD74). No changes in gene expression were observed which might be linked with toxicity, i.e. genes associated with apoptosis, stress, and enzymatic induction. In conclusion, the genomic analysis of blood from psoriasis patients treated with oral SDZ ASM 981 indicates a broad anti-inflammatory activity without evidence of toxicity. The mRNA expression changes are thus consistent with the observed clinical efficacy and safety. To the best of our knowledge, the gene expression analysis reported here is the first of this kind in dermatological diseases.

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Tazarotene 0.1% Gel in the Treatment of Fingernail Psoriasis: A Double-Blind, Randomized, Vehicle-Controlled Study

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To compare the efficacy and tolerability of once-daily tazarotene 0.1% gel and once-daily vehicle gel, both occluded and nonoccluded, in the treatment of fingernail psoriasis. (Tazarotene is not approved for the treatment of fingernail psoriasis.) Patients eligible for recruitment were adults with psoriasis affecting at least 2 fingernails that was characterized by at least 3 of the following: pitting, onycholysis, subungual hyperkeratosis, leukonychia, nail plate crumbling and/or nail plate loss, splinter hemorrhages, and nail bed discoloration. Patients were randomized to receive tazarotene gel or vehicle gel, to be applied to each target fingernail and the surrounding nail folds every evening for up to 24 week. One target fingernail was occluded with polyethylene film/sheeting during the treatment period. No other medications were allowed on the fingernails. Data are available from 28 patients to date (18 in tazarotene group, 10 in vehicle group). For nonoccluded nails, the tazarotene regimen was consistently more effective than vehicle in reducing onycholysis ($p = 0.05$ at Wk 12). For occluded nails, the tazarotene regimen was significantly more effective than vehicle in reducing pitting ($p = 0.05$ at Wk 24). There were no other significant between-group differences in onycholysis, pitting, subungual hyperkeratosis, leukonychia, nail plate crumbling/loss, splinter hemorrhage, or nail bed discoloration in nonoccluded or occluded nails. Three patients (all tazarotene group) reported treatment-related adverse events (peeling of proximal nail fold skin, irritation of skin on finger, and erythema of proximal nail fold). All such adverse events were mild. Applications of tazarotene 0.1% gel to psoriatic fingernails and the surrounding nail folds can significantly reduce onycholysis (in nonoccluded nails) and pitting (in occluded nails). This information in part will be also submitted to the AAD.

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Randomized Double-Blind Placebo-Controlled Trial Using Interleukin-10 For Psoriasis

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Psoriasis lesions are characterized by high levels of pro-inflammatory and type 1 cytokines (e.g. TNF-α, IFN-γ). Because of this, several investigators have reported on the open-label use of recombinant human interleukin 10 (rhIL-10), a type 2 cytokine, in psoriasis patients. Here, we performed a 12- week randomized double-blind placebo-controlled trial using rhIL-10 in patients with moderate-to-severe psoriasis (PASI scores 10). 28 patients who were off all other psoriasis medications were enrolled; 26 were subsequently considered statistically evaluable. 2/3 of patients received 20 μg per kg rhIL-10 and 1/3 received placebo injected SC 3X/ week. The primary endpoint was the PASI score at week 12. There were no significant differences in the change in PASI scores from baseline to week 12 between the rhIL-10 group and control patients (mean percentage change in PASI = 17% and 13%, respectively; $p = 0.69$). However, a strong trend toward improvement was noted in patients receiving rhIL-10 vs. placebo at 6 week (32% vs. 7%, respectively; unadjusted $p = 0.057$) and at 8 week (36% vs. 13%, respectively; unadjusted $p = 0.027$). Pancreatitis occurred in 1 rhIL-10-treated patient, but was well tolerated by the other patients. Declines in hemoglobin at 2 week ($p = 0.0078$), platelet counts at 1 week ($p = 0.00034$), and cholesterol at every time point were observed in the rhIL-10 group. Intracellular cytokine staining of PBMC from the rhIL-10 treated patients revealed progressive declines in the IFN-γ/IL-4 ratio and TNF-α production in CD4+ T cells, as well as decreases in TNF-α production in monocytes, over the 12-week period. In summary, we found that rhIL-10 was well tolerated by most patients, induced distinct hematologic and immunologic changes, and demonstrated significant antipsoriatic effects for 6–8 week following onset of treatment. However, we conclude that rhIL-10 is not useful as a treatment option for psoriasis patients because clinical responses were not maintained, despite clear shifts in the type 1/type 2 cytokine profile in PBMC.

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Alefacept Treatment for Psoriasis Reduces the Number of Infiltrating IFNγ+-Producing T cells in Lesional Skin

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Psoriasis is a chronic inflammatory skin disease mediated, in part, through IFNγ production by activated lesional T cells (Th1 skewed). Alefacept (human LFA-3/IgG1 fusion protein, LFA3TIP, currently being developed under trade name AMEVIVE(tm)) is a novel recombinant protein that leads to a reversible reduction in CD3+CD45RO+ T cells. To study effects on skin T cells, an open-label phase 3 study was conducted in 6 patients with psoriasis who received alefacept 7.5 mg IV once weekly for 12 consecutive weeks. The percentages of CD3+ T cells and IFNγ-producing CD3+ populations in total epidermal or dermal cells were analyzed by flow cytometry, and CD3+ or IFNγ+CD3+ cell densities as cell number/mm^2 were calculated. In the 5/6 patients demonstrating clinical improvement at week 13, the density of epidermal T cells producing IFNγ (IFNγ+CD3+) was reduced to 0.26 ± 0.3% of baseline. The mean density of IFNγ+CD3+ cells for all 6 patients at baseline was 182 ± 91 per mm^2 vs. 77 ± 45 per mm^2 after 12 weeks of alefacept treatment ($p = 0.05$). For all 6 patients, PASI improvement correlated with percentage change in IFNγ+CD3+ epidermal cells with $r = 0.80$ ($p = 0.06$). Interestingly, in the initial phase of treatment, several patients demonstrated transient increases in IFNγ+CD3+ lesional T cells at week 3, in association with a transient increase in epidermal thickness; both the IFNγ+CD3+ T cells and epidermal thickness then decreased in subsequent weeks. Our results suggest that alefacept can significantly reduce the number of infiltrating Th1-type IFNγ+CD3+ T cells, in association with clinical improvement. Because IFNγ is believed to be a key factor in psoriasis pathogenesis, the reduction of Th1 cells in the skin may represent a critical step in the clinical improvement of psoriasis.

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IL-10 Therapy in Psoriasis: Effects on the Skin Immune System

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With its anti-inflammatory and immunosuppressive properties interleukin (IL)-10 plays an outstanding role in several immune reactions including regulatory mechanisms in the skin. Recently, we performed a phase II trial in 10 psoriatic patients, receiving subcutaneously recombinant human IL-10 over a 7-week period. The clinical response (PASI decrease over 50%) suggested that IL-10 might represent a novel antipsoriatic drug. In order to better understand the mode of action and to elucidate the effects of systemic IL-10 treatment on the skin immune system, skin punch biopsies were analysed, obtained from the patients before (day – 6), at the end (day 50), and 3 weeks after IL-10 therapy (day 71) during this study. Histological examination showed a decrease of several parameters reflecting the psoriatic disease activity as acanthosis and extension of the horny layer. Immunohistological examination demonstrated decreasing numbers of infiltrating epidermal and dermal T-cells, dermal CD1a+ cells, and a diminished proliferation of epidermal cells. Using a novel, quantitative RT-PCR approach a significant shift within the cytokine pattern was found. So IL-10 therapy lead to an increase of cutaneous IL-4 and decrease of IL-8 and IL-10 mRNA expression. No significant changes within of IL-2, IL-6, TNF-α, and IFN-γ expression were found. So a shift from a type1 towards a type 2 cytokine pattern. The changes within the local cytokine pattern seems to be disease related, since an inverse course was found in the single IL-10 nonresponding patient. Our findings demonstrate the considerable effects of systemic IL-10 application on the skin immune systems. Those might contribute to the antipsoriatic activity of IL-10.

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IL-10 Therapy in Psoriasis: Systemic Imunological Effects

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A clinical phase II trial with long-term (49 days) subcutaneous administration of recombinant human IL-10 revealed clinical efficiency in 9 of 10 psoriatic patients. Despite temporary very high systemic IL-10 levels (500 pg per ml) only moderate signs of immunodepression and no infectious complications were observed. So, IL-10 treatment caused transient falls in monocytic HLA-DR expression and proinflammatory cytokine secretion capacities (TNF-α, IL-1β, IL-12) as well as in IL-12 plasma concentrations. Interestingly, monocytic expression of CD64 and transferrin receptor as well as the plasma levels of IL-6, C-reactive protein, soluble IL-2R and the blood sedimentation rate increased in parallel indicating monocytic activation/differentiation and an acute phase response. For lymphocytes, decreases of T-cell HLA-DR expression and NK-cell numbers and increased plasma IgE levels were found. In contrast to these transient changes during IL-10 therapy, other alterations persisted or even further strengthened in the post-theraeutic 7-weeks observation period. We observed strong increases of T-cell IL-4 and NK-cell IFN-γ production capacities as well as rising numbers of CD5+ B-cells indicating lasting re-regulation of the immune system. Moreover, persistently decreased expression of monocytic accessory molecules in parallel with increased IgA and decreased TGF-β1 plasma levels indicated an enhanced TGF-β utilisation as one mechanism of lasting immunomodulation. From these results we hypothesize that IL-10 may have the capability to lastingly improve the immunopathophysiological situation in psoriasis

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Comparison of the National Psoriasis Foundation Psoriasis Score with Psoriasis Area and Severity Index

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The National Psoriasis Foundation (NPF) Medical Advisory Board has developed a new NPF Psoriasis Score (NPF-PS) to measure clinically significant improvement as a function of psoriasis treatment. This dynamic, five-component method, which incorporates a quality of life measurement, has been proposed in response to the limitations of the Psoriasis Area and Severity Index (PASI) including its reliance on imprecise estimates of the involved Body Surface Area (BSA). The equally weighted primary endpoints of the NPF-PS, which contribute a score range of 0–30, include an assessment of induration of two target lesions, BSA, Physician's and Patient's Global Assessments, and itching. We compared the performance of the NPF- PS with the PASI in an investigator-initiated, single site, randomized, double-blind, placebo-controlled clinical trial of infliximab (an anti-TNFα monoclonal antibody) in patients with moderate to severe plaque psoriasis. Thirty-three patients were randomized to receive infliximab infusions at 5.0 mg per kg, 10.0 mg per kg or placebo in a 1:1:1 fashion. For purposes of this comparison, patients were considered responders based on the NPF-PS and based on the PASI if there was a = 75% improvement in the score. Patients were pooled across the three treatment groups for this comparison. The Spearman rank correlation was used to assess the linear association between the scores. The Kappa statistic was used to determine the agreement among the categorical outcome of responder status using the two scores. The three patients that withdrew during the course of the study (one from each group) were considered as nonresponders. The raw scores at each time point (biweekly from week 0–10) and the percentage improvement in scores during treatment compared to baseline (Week 2–10) showed a moderate linear correlation. Incorporating all time points ($n = 187$), the Spearman correlations between NPF-PS and PASI were 0.87 (raw scores) and 0.93 (% improvement). At week 10, the Kappa statistic of 0.94 ($n = 33$) showed an "almost perfect" agreement between NPF and PASI in differentiating responders. In this therapeutic trial, the NPF-PS showed a significant association with the PASI in measuring clinically significant outcomes. To date, this is the first report of a double-blind, placebo controlled study that demonstrates an association between the NPF-PS and PASI.

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Comorbidity Incidence of Acne and Depressive Disorders in Isotretinoin Users Versus Non-users: A Retrospective Analysis of the National Ambulatory Medical Care Survey Database

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Isotretinoin-induced depression in acne patients has been anecdotally reported, but in a recent population-based study this association was not supported. We determined the incidence of office visits for outpatients diagnosed with concurrent acne and depression in order to assess the proportion attributable to isotretinoin exposure. Using a nationally representative database, an estimated 85 000 visits occurred at which acne and depression were diagnosed together between 1995 and 1998. None of these events were observed in patients treated with isotretinoin.

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Cost Analysis of Isotretinoin for Treatment of Severe Acne

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Objective: A retrospective analysis of administrative claims data was performed to evaluate the impact of a single course of isotretinoin therapy on the direct cost of medical treatment for severe acne. Data and Methods: Using the MEDSTAT Marketscan database, we identified 475 subjects aged 12-35 who received a single course of isotretinoin lasting 90 days or longer from 1995 through 1998. Acne-related expenditures were calculated during isotretinoin treatment and for periods of 12 months before and after isotretinoin use. Direct costs, including cost of isotretinoin, systemic and topical antibiotics, laboratory tests, and doctor office visits, were examined. The analyses included univariate statistics and multivariate analyses to evaluate how healthcare expenditures were affected by individual subject characteristics such as gender, age, and health plan. Results: On average, subjects received isotretinoin for 141 days at a cost of $1231. Mean total acne-related expenses declined from $471 (median, $311) per year prior to isotretinoin therapy to $135 (median, $48) per year after treatment ($p < 0.01$). More than 25% of subjects had no acne-related expenditures for at least 12 months following isotretinoin therapy. Conclusion: Isotretinoin therapy substantially reduces direct costs for acne-related medical care. Direct costs associated with over-the-counter preparations and unreimbursed expenses (e.g., for cosmetic procedures) were not included. Furthermore, indirect costs of therapy for severe acne, including travel and time away from school/work for doctor's appointments, and value attributed to changes in quality of life were not included. Inclusion of such costs and value would likely substantially increase the calculated cost-benefit of isotretinoin in the treatment of severe acne.

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Use of Accutane and Treatment for Depression: A Prescription Sequence Symmetry Analysis

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Objective: Data from a large sample of patients with incident use of both Accutane and antidepressants were reviewed to calculate, within study period, the ratio of asymmetry and the incidence rate ratio of antidepressant prescriptions in Accutane-treated patients to determine if there may be a causal relationship between Accutane use and depression. Secondary objective was to evaluate validity of Accutane analysis using Minocycline, an agent not suspected to cause depression. Methods: Prescription sequence symmetry analysis (PSSA) was successfully used by Hallas (*Epidemiology* 7:478-484, 1996) to demonstrate possible causal relationship between certain beta blockers and antidepressant use. Applying PSSA, analysis of dispensing patterns of Accutane and antidepressants was performed using Synergy Healthcare, a large U.S. claims database. Prescribing order of Accutane and antidepressants was determined to identify any possible association between Accutane and depression. Ratio of asymmetry was computed as the ratio of number of patients prescribed antidepressants second over number of patients prescribed antidepressants first. Antidepressants were classified by class (amines, SSRIs, ''other'') or in aggregate. Data were reviewed from July 1998 through March 2000, and waiting period analysis to determine incidence use restricted study period to June 1999 through March 2000. Results: Of 90 million patients represented in Synergy pharmacy file, 5,753,565 patients filled prescriptions for Accutane and/or an antidepressant. Of those, 17,348 patients filled prescriptions for both Accutane and antidepressants yielding 2,821 incident users of both drugs during study period. Resulting ratios of asymmetry were 0.99 (95% CI = 0.93-1.06) for selective serotonin reuptake inhibitors (SSRIs), 0.90 for amines (95% CI = 0.78-1.04), 0.95 (95% CI = 0.85-1.05) for ''other'' antidepressants, and 0.97 (95% CI = 0.92-1.02) for all antidepressants combined. Results suggest little to no effect on sequence of Accutane and antidepressant prescribing order when analyzed by antidepressant class or in aggregate. Conclusion: Results of these analyses show no significant asymmetry in prescription order of Accutane and antidepressants. This provides evidence against casual association between use of Accutane and treatment for depression. In addition, results obtained from Minocycline analysis confirmed reliability of Synergy database and validity of PSSA methodology.

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Treatment of Inflammatory Acne with an Oral 5-Lipoxygenase Inhibitor

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Peroxisome proliferator-activated receptors (PPARs) have recently been shown to be involved in the differentiation of the sebaceous gland cells. Regulation of PPARα has been associated with a pro-/anti-inflammatory action and lipid catabolism. Leukotriene B_4 (LTB_4), a 5-lipoxygenase metabolite of arachidonic acid, is a ligand for PPARα. Therefore, an ethic committee-approved, industry-independent, clinical proof of principle (1C) study was conducted to treat inflammatory acne with an oral selective 5-lipoxygenase inhibitor. Ten consecutive patients with acne papulopustulosa (m:f 6:4, aged 19 ± 5 years) were treated with (+/–)-1-(1-benzo[b]thien-2-ylethyl)-1-hydroxyurea 4 × 600 mg per day p.o. for 3 months after providing written consent. Clinical evaluation by counting of lesions and determination of overall severity index after Allen and Smith, assessment of casual skin surface lipids by Sebumeter(r) and of liver enzymes in serum were performed at baseline, weeks 2, 4, 8, 12 of treatment and a 2-week post-treatment period. LTB_4 in blood by radio-immunoassay and sebum lipid fractions by gas chromatography were determined at baseline and on week 12. Patients were photographed before and at the end of treatment. The acne severity index continuously decreased in a time-dependent manner being 41 ± 28% of the initial score at week 12 ($p < 0.05$). This was due to a decrease of the number of inflammatory lesions to 29 ± 24% ($p < 0.01$), while comedones did not respond to treatment. Neither subjective nor objective adverse events were registered. In addition, total sebum lipids significantly decreased (35 ± 51%, $p < 0.05$) and the pro-inflammatory free fatty acids (22 ± 18%) and lipoperoxides (26 ± 30%) were markedly diminished in patients' sebum under treatment. The magnitude of clinical improvement strongly correlated with the reduction of total sebum lipids ($p = 0.0009$, $r^2 = 0.81$) and free fatty acids ($p = 0.0003$, $r^2 = 0.82$). In contrast, LTB_4 levels in blood and the casual skin surface lipids were not affected. All parameters studied remained practically unchanged during the 2-week post-treatment observation. In conclusion, we could provide first indirect evidence that acne is a genuine inflammatory disorder. Inflammatory acne lesions significantly responded to an oral 5-lipoxygenase inhibitor. Moreover, systemic inhibition of arachidonic acid metabolism decreased total sebum lipids and pro-inflammatory lipid fractions in sebum, which are considered to be responsible for the development of inflammatory acne lesions.

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The Efficacy of a Low-Dose Oral Contraceptive for the Treatment of Moderate Acne and its Effects on Biochemical Markers of Androgenicity: Two Randomized, Placebo-Controlled Trials

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The purpose of these studies was to evaluate the efficacy of a low-dose oral contraceptive (OC) containing ethinyl estradiol 20 μg/levonorgestrel 100 μg (EE/LNG) for the treatment of moderate acne and its impact on biochemical markers of androgenicity. Data from two randomized, double-blind, placebo-controlled, multicenter trials were pooled and analyzed. Healthy females ($n = 721$; ≥ age 14) with regular menstrual cycles and moderate facial acne were randomized to receive EE/LNG or placebo for 6 cycles of 28 days. Primary efficacy endpoints included inflammatory, noninflammatory, and total lesion counts; data analyzed for these endpoints included the last observation carried forward for all patients. Ovarian, adrenal and peripheral androgens, and sex hormone-binding globulin (SHBG) were also measured and analyzed in a subset of patients ($n = 30$). At the end of the study, EE/LNG significantly reduced the number of total lesions from baseline compared to placebo ($p < 0.01$). There was also a significantly lower number of inflammatory and noninflammatory lesions in EE/LNG patients compared to placebo patients ($p < 0.05$). After 6 cycles of treatment, significant decreases in ovarian (androstenedione, free testosterone, bioavailable testosterone) and peripheral (3α-androstanediol glucuronide, androsterone glucuronide) androgen levels, and a significant increase in SHBG were observed with EE/LNG compared to placebo ($p < 0.05$). Compared to baseline, EE/LNG significantly reduced free testosterone, bioavailable testosterone, and 3α-androstanediol glucuronide, and significantly increased SHBG at cycle 6 ($p < 0.05$). Significant positive correlations were found between changes in total lesions and changes in androstenedione, free testosterone, and dehydroepiandrosterone sulfate levels ($p < 0.05$), and a trend toward a significant ($p = 0.0595$) inverse correlation was found between changes in total lesions and changes in SHBG levels. In conclusion, this low-dose OC, Alesse(r), containing 20 μg EE/100 μg LNG, is effective and safe for the treatment of moderate acne and improves biochemical markers of androgenicity.

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Topical Azelaic Acid in the Treatment of Rosacea

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Topical 20% azelaic acid cream was compared with placebo in a nine-week, randomized, controlled trial at a large multispecialty clinic in the upper Midwest. Subjects with rosacea were recruited over a one-year period, excluding those who were under 25 years of age, pregnant, or lactating, or had recently used topical or oral medications that affect rosacea. A total of 60 subjects met study criteria and provided informed consent. Baseline assessment of subjects' rosacea included papules/pustules, erythema (5 sites) and telangiectasia (5 sites). Subjects were randomized to treatment and control groups, and were instructed to apply cream – azelaic acid or placebo – twice daily for the duration of the nine-week trail. Fifty-three subjects completed the trial. Post-treatment assessments were conducted on day 63. For each variable, baseline and post-treatment assessments were compared to determine if the subject was improved, unchanged, or worse after treatment. Azelaic acid treatment was associated with improvement in papules/pustules ($p < 0.05$). No other significant difference between azelaic acid and control regimens was noted. These findings confirm the reported efficacy of azelaic acid in the treatment of the papulopustular form of rosacea. Additional work is needed to clarify the role that azelaic acid may have in the treatment of the full spectrum of clinical presentations of rosacea.

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Application of Dermatopharmacokinetic Approach in the Assessment of Clinical Bioequivalent And Bio-inequivalent Tretinoin Gel Products

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Dermatopharmacokinetics (DPK) has been proposed as a method for bioequivalence (BE) assessment of locally acting topical drug products. Multiple adhesive discs are used to harvest stratum corneum (SC) from treated skin sites at various time points after product application and removal. Three 0.025% tretinoin gels: Retin-A(r) gel (Ortho), Tretinoin gel (Spear) and Avita(r) gel (Bertek) were compared for drug uptake into and elimination from forearm SC in 49 healthy subjects, using a 5-μL dose/1.13 sq cm surface area skin site. One skin site was used for each time point and each gel evaluated. The products were applied simultaneously, side-by-side, in each subject. SC from each skin site was harvested by a validated skin stripping method. Tretinoin and isotretinoin concentrations were determined in the SC samples by a validated HPLC method. Cmax of tretinoin and isotretinoin with the Ortho gel was 115 and 55 ng/sq cm. Tmax was 0.7 and 0.8 h, respectively. Elimination half-life was 5.6 and 9.4 h, respectively. AUC0-t was 430 and 303 ng- h/sq cm, respectively. The DPK BE results with these gels concur with the previous clinical findings. Thus, differences between Spear and Ortho gels, that are clinically bioequivalent and qualitatively/quantitatively the same in composition, were smaller than between Bertek and Ortho/Spear gels, which are clinically bio-inequivalent and differ in composition. These data demonstrate that clinical and DPK approaches produce the same BE assessment of three 0.025% tretinoin gels and validates DPK as an appropriate method for BE assessment of topical drug products.

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Food Effect on a New Formulation of Accutane

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Purpose. Isotretinoin, 13-cis-retinoic acid, is approved for the treatment of severe recalcitrant, nodular acne. The currently marketed formulation (Accutane,) must be administered with food for optimal extent of absorption. A new formulation of isotretinoin was developed to minimize the food effect and increase bioavailability. Methods. A 4-way crossover PK study was conducted in 87 (1F/86M) healthy adult subjects to assess the effect of food on a single oral 30 mg dose of the new formulation compared with 80 mg of marketed Accutane under fed and fasted conditions. Plasma concentrations were assessed for isotretinoin and its 3 major active metabolites. Results. The primary PK parameters (mean±SD) of isotretinoin are: New Fed: AUC_{0-o} 5469 ± 1291 ng*h per mL, C_{max} 448 ± 164 ng per mL; New Fasted: AUC_{0-o} 4183 ± 1140 ng*h per mL, C_{max} 336 ± 122 ng per mL; Marketed Fed: AUC_{0-o} 10004 ± 2198 ng*h per mL, C_{max} 862 ± 344 ng per mL; Marketed Fasted: AUC_{0-o} 3703 ± 1704ng*h per mL, C_{max} 301 ± 191 ng per mL. These data show that for the new formulation, the mean AUC_{0-o} increased by ~30% under fed conditions; for marketed isotretinoin, mean AUC_{0-o} increased by ~200% under fed conditions. Similar results were seen with the 3 major metabolites. Conclusions. The study shows that the new formulation can be administered with or without food.

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Dose Proportionality of New Formulation of Accutane

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Purpose. Isotretinoin, 13-cis-retinoic acid, is approved (Accutane,) for the treatment of severe recalcitrant, nodular acne. A new formulation was developed to minimize the food effect on isotretinoin and increase the bioavailability. Methods. A single oral dose, 3-way crossover PK study was conducted in 42 healthy male subjects to establish the dose proportionality of 2 × 7.5 mg, 2 × 15 mg, and 2 × 22.5 mg capsules of the new formulation. Pharmacokinetics were assessed for isotretinoin and its 3 major active metabolites. Results. The mean ratios for dose-normalized AUC_{0-o} (ng* h per mL) and C_{max} (ng per mL) are as follows: Isotretinoin AUC_{0-o} Ratio for 15:30 mg: 111%; for 45:30 mg: 90%. Isotretinoin C_{max} Ratio for 15:30 mg: 105%; for 45:30 mg: 96%. 4-oxo-isotretinoin AUC_{0-o} Ratio for 15:30 mg: 101%; for 45:30 mg: 89%. 4-oxo-isotretinoin C_{max} Ratio for 15:30 mg: 86%; for 45:30 mg: 92%. Tretinoin AUC_{0-o} Ratio for 15:30 mg: 92%; for 45:30 mg: 84%. Tretinoin C_{max} Ratio for 15:30 mg: 85%; for 45:30 mg: 105%. 4-oxo-tretinoin AUC_{0-o} Ratio for 15:30 mg: 90%; for 45:30 mg: 97%. 4-oxo-tretinoin C_{max} Ratio for 15:30 mg: 106%; for 45:30 mg: 92%. Conclusion. The data show that the exposure to isotretinoin and its 3 major active metabolites is dose proportional within the range of 15 mg to 45 mg.

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Development of New 5-α-Reductase Inhibitors

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5-α reductase, the enzymes system that metabolizes testosterone into dihydrotestosterone (DHT), occurs in two isoforms, the type A which represents the ''cutaneous type'' and the type 2 which is located mainly in the epididymis, seminale vesicles, prostate,…Androgen-dependent skin disorders, such as seborrhoea, acne, female hirsutism and/or androgenetic alopecia are among the most common diseases encountered by the dermatologist. Thus the development of new compounds that effectively inhibit the type 1 isoenzyme of 5-α reductase are of major importance for dermatological purpose. The aim of this work is to present new compounds which are able to inhibit significantly 5-α reductase type 1. The efficiency of these molecules was tested on human skin fibroblasts in culture. Cells were cultured with radiolabelled testosterone and the evaluation of DHT formation by thin-layer chromatography has been applied to evaluate 5-α reductase activity. Fatty esters and natural extracts have been tested. Concerning fatty compounds, a maximal inhibitory effect is observed in the presence of mono unsaturated C18 long chain butyl ester and/or ricinoleic group. These results emphasize the potentiel benefit of butyl avocadate and methyl ricinoleate as 5-α reductase inhibitors and their application for the development of cosmetic and pharmaceutical products dedicated for the treatment of dermatological disorders such as acne, hirsutism and alopecia.

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R115866: A Selective Retinoic Acid Metabolism Inhibitor Acts Topically as a Retinoidal Agent in Cutaneous Model Systems

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R115866 is a highly potent and selective inhibitor of P450-mediated all-*trans*-retinoic acid (RA) metabolism. This compound has been shown to exhibit retinoidal effects when given orally. The purpose of our studies was to profile R115866 for topical retinoid activity in a variety of cutaneous models. In rhino mouse skin, a model of follicular keratinization, R115866 was two times less potent than RA at reducing utriculus size, but both compounds were equiactive at 0.03%. R115866 at 0.003, 0.01 and 0.03% was also effective at causing connective tissue repair in photodamaged hairless mouse skin. In a mouse model of glucocorticoid-induced skin atrophy, R115866 at 0.03%, like RA, completely prevented skin thinning. R115866 also induced hyperplasia of the epidermis in swine (0.1% dose) and xenografted human skin (0.03% dose). In the ear of the Abyssinian × Shorthair guinea pig, R115866 reduced the amount of epidermal melanin in a dose-related fashion (0.01–0.1%). In the rabbit dermal irritation model, R115866 caused minimal irritation (erythema) at 0.1%, compared to RA at the same dose, which was irritating. These findings suggest the use of R115866 as a new topical dermatotherapeutic agent for treating retinoid-responsive skin disorders including acne, photodamage, psoriasis and hyperpigmentation.

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***In Vivo* Evaluation of an Antiaging Cosmetic Product Containing both Avocadofurane and Soy Isoflavones Using 31P Magnetic Resonance Spectroscopy of the Skin**

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The cutaneous aging process includes intrinsic physiological factors and extrinsic environnemental factors mainly related to solar irradiation. After menopause, hormonal deprivation of sexual steroids due to the loss of ovarian activity plays an important role in intrinsic aging. Clinically, menopausal women present signs of dry, thin and pale skin which are related to the. We have developped a cosmetic product, containing both avocadofurane (1%) and soy isoflavone, which is specifically dedicated to the treatment of hormonal skin aging. 31P nuclear magnetic resonance spectroscopy is a non invasive tool to quantify energy metabolism of human skin via the measurement of the main phosphorylated metabolites: inorganic phosphate (Pi), phosphocreatine (PCr) and adenosine triphosphate (ATP). The aim of this work was to evaluate the changes in the energy metabolism of the skin, after application of antiaging product (Helyx+, Noviderm). Subjects applied the product twice daily on one wrist (the other was taken as control). Spectra of the skin of both wrist were acquired at T0, T + 4, T + 7 h to assess short-term effects, and at T + 14 days (evaluation of a remanent effect). Treatment with Helyx + results in a significant short (PCr/Pi + 9.1% at T + 3 h) and long-term (ATP/Pi + 16.9% at T + 14 days) improvement in the energetic status of the skin. This study allow us to conclude that Helyx + is an energizing cosmetic product specifically designed for the treatment of hormonal skin aging. Indeed the association of both avocadofurane, which is able to induce TGF-β1 and collagen synthesis, and soy isoflavones may contribute to the restoration of the energetic status of aged skin and by the way to the improvement of the visible signs of hormonal cutaneous aging.

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Clinical Assessment of Topical Tissue-Engineered Growth Factors for Reversal of Photodamaged Skin

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A clinical evaluation was conducted with 12 moderately wrinkled patients who were screened to have type (4 thru 6) wrinkles as defined by the Fitzpatrick scale. Patients were treated topically with formulations containing naturally secreted skin growth factors including VEGF, KGF and TGFβ. Preliminary data analysis of a previous pilot study showed a 10–30% increase in epidermal thickening and a 20–60% increase in Grenz zone collagen after 30 days of daily application. In the current evaluation, dose ranging studies were performed with a clinically relevant target dose of TGFβ (16 ng per ml) and sequentially reduced dosing. 60-day biopsies are pending and will be assessed. Patients received biopsies of the cheek on the first office visit and the last office visit, which was at the end of the 60-day evaluation. Subjects also received surface profilometry testing of wrinkles on the initial office visit and the last office visit. Patients treated the entire face twice daily for 60 days and returned for evaluation at four-week intervals. 60 day biopsies are pending and will be assessed for increases in Grenz zone collagen and epidermal thickening Clinical responses were also analyzed utilizing clinical ratings of the Fitzpatrick scale as well as assessment of optical profilometry. While there were no controls in this evaluation, the assessment from baseline demonstrated that the topical tissue-engineered growth factor used twice daily results in both remodeling and rejuvenation of skin.

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Molecular Transporters Facilitate Topical Protein Transduction into the Skin

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The skin provides a formidable barrier to delivery of molecular therapeutics. Recently, new molecular transporters in the form of protein transduction sequences (PTS) derived from proteins such as HIV1 TAT and Drosophila antennapedia have been found to rapidly traverse cell and visceral tissue barriers, including the blood–brain barrier. Carrying cargo up to 40 nm in size, these transporters appear to rely on arginine residues that are also present in evolutionarily conserved sperm protamines that facilitate delivery of macromolecules such as the male haploid genome. To determine if these PTS could transport cargo across the cutaneous permeability barrier, we have recently characterized successful transdermal delivery of cyclosporin A conjugated to arginine heptamers. To further investigate the use of PTS for transdermal delivery and to define optimal transporters, we designed a series of oligoarginine PTS ranging in size from R7 to R10 and conjugated them either to a peptide with biological function or a biotin label. Additionally, similar constructs with a glycine-spaced heptarginine as well as transporter sequences from TAT and antennapedia were constructed in parallel. A disulfide linkage to PTS was designed that releases functional cargo upon entry into the reducing environment within the cell. We compared the toxicity and ability of this array of transporter sequences to carry their cargo into keratinocytes in culture as well as across the skin barrier *in vivo*. We found that PTS alone have varying levels of toxicity when applied to cultured human keratinocytes. At concentrations of 100 μM to 0.1 μM the decamer of arginine was found to display keratinocyte toxicity. Over the same range of concentrations, a heptamer of arginine was less toxic and the TAT PTS was nontoxic. The level of toxicity was roughly correlated to the effectiveness of the transporter. We demonstrated efficient transportation of FITC labeled PTS into keratinocytes *in vitro* and with biotinylated PTS conjugated peptides to intact mouse skin *in vivo*. Therefore, it appears that arginine-based PTS are generally effective transporters into keratinocytes in culture and across the cutaneous barrier *in vivo*. This new molecular transport technology thus represents a promising new platform for topical delivery of therapeutics to skin.

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The Skin as a Bioreactor: A Transgenic Mouse Model with Regulated Cutaneous Delivery into the Central Circulation

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Epidermal keratinocytes secrete a large number of polypeptides, and some of these have been shown to achieve systemic distribution via the vascular system within the dermis. This raises the possibility that the epidermis may be used as a bioreactor to produce proteins for systemic release to treat disease. The initial reports documenting the feasibility of this approach utilized vectors that directed constitutive expression of therapeutic genes. Expression of a therapeutic gene usually must achieve a threshold level in order to have therapeutic effects, however, constitutive expression of a transgene at high levels may be deleterious to the cells and organism. To circumvent these problems, inducible vector systems have been developed which allow regulated expression of transgenes. To test the feasibility of using these inducible vectors *in vivo* in the skin, we have developed a transgenic mouse model in which expression of the transgene can be regulated temporally by topical induction. In this system, a transcription activator driven by an epithelial-specific promoter remains inactive until topical application of an inducer. Upon induction, the activator activates transcription of the therapeutic gene, human growth hormone (hGH), in basal keratinocytes. High levels of hGH were detected in the circulation within hours after a single application of the inducer. Maximum hGH levels (~1000-fold higher than controls) were observed a few days later, with the serum levels gradually returning to base line. Repeated induction resulted in up to 45% weight gain in adult transgenic mice, compared with 7% in control mice. We also demonstrated that the serum levels of hGH were dependent on the amount of inducer applied. To simulate how this inducible system might eventually be used in the clinic, skin biopsies from these transgenic mice were grafted onto nude mice. Following induction of the grafted area, physiological levels of hGH were detected in the serum. These results clearly demonstrate the feasibility of regulating the delivery of therapeutic proteins into the circulation via genetically modified keratinocytes.

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Sustainable Systemic Erythropoietin Delivery via a Single Injection of Lentivirus into Human Skin

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The skin offers an attractive tissue site for systemic gene therapy because of its accessibility and ability to deliver proteins to the bloodstream. Among systemic disorders potentially amenable to cutaneous gene transfer are those characterized by inadequate blood levels of specific polypeptides, such as is seen in erythropoietin (EPO)-responsive anemia. In an effort to achieve systemic EPO delivery via cutaneous gene transfer, we generated a VSV-G pseudotyped third generation HIV-1-based lentiviral vector encoding human EPO for delivery to full thickness human skin xenografts on SCID mice. Intradermal injection of 1, 5 and 10 μg p24 equivalent [n = 5 mice/group] produced dose-dependent levels of human EPO of 5, 11 and 35 mIU/ml [control = 0] and hematocrit of 49, 54 and 65 [control = 40], respectively. EPO expression and increased hematocrit are stable out to at least 10 months after a single injection. To determine if the infectious virus was only localized to skin rather than disseminated to visceral tissues, skin biopsy of the injection sites of mice injected with 1 and 10 μg of lentivector was performed at 4 weeks. Within days after removal of the injected skin tissue site, blood EPO fell to undetectable levels, confirming that the skin was the only site of infection and EPO production. Double immunostaining of human skin to identify the skin cell types targeted revealed that lentivectors effectively transduce keratinocytes, fibroblasts, macrophages and endothelial cells of skin within the area closely localized around the site of needle injection. 3343 + 1438 cells of all types within skin were transduced per mcg p24 equivalent of the EPO lentivector, indicating that a as few as 30 000 EPO-expressing cells within skin may suffice to boost hematocrit over 60%. Thus, lentiviral vectors are capable of sustained delivery of a therapeutic polypeptide to the bloodstream after a single injection with localized infection of multiple cell types within skin and provide a promising approach for future cutaneous gene transfer.

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Physiological Effect of ''Green Odor'' on the skin

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''Green Odor'' is a natural fragrance we feel fresh and green in and around the forest. To clarify the relationship between ''Green Odor'' and skin conditions, we used leaf-alcohol (3Z-hexenol) and leaf-aldehyde (2E-hexenal) as ''Green Odor''. We studied 5 patients with atopic dermatitis (one male and 24 female, 21–31 year) and 87 normal healthy controls (63 males and 24 females, 22 to43 year). In a room with temperature kept at 23°C and 60% humidity, the subjects sat comfortably for 30 min, smelling leaf-alcohol and leaf-aldehyde both diluted to 1% with solvent (triethyl citrate). Before and after their smelling, we measured the following three points: (1) skin temperature of both hands with thermography (2) water content of the stratum corneum in both hands with Skicon-200, and (3) blood pressure, pulse rate, and body temperature. Estimations of all experiments were indicated as ratio of the value of after-smelling/the value of before-smelling. Two of the 5 patients with atopic dermatitis showed decrease of the skin temperature and increase of the water content of the stratum corneum, while no obviously detectable change was observed in the 87 normal healthy controls. We had patch tested ''Green Odor'' on 93 normal healthy controls and one patient with atopic dermatitis and had confirmed none of them had shown positive reaction. In conclusion, it seems that ''Green Odor'' may contribute to the improvement of the physiologically aberrant skin conditions of patients with atopic dermatitis.

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Doxycycline-Inducible Retroviral Expression of Green Fluorescent Protein in Immortalized Human Keratinocytes

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Ease of culture, potential for transplantation and reported success of expression of transgenes are features that make keratinocytes attractive for localized or systemic delivery therapeutic genes. Insertion of a regulatable switch for transgene expression into keratinocytes would greatly enhance their clinical utility for gene therapy. We describe a method wherein immortalized human keratinocytes (IMKc) that have been shown to form a functional epidermis *in vivo* can be transduced with high efficiency with the retroviral vectors of the RetroTet-Art system which confers stable doxycycline (Dox) inducible green fluorescent protein (GFP) expression. This system houses a transactivator and a transrepressor of the transgene which are activated with the tetracyclines. After one round of transduction, approximately 50% of IMKc expressed GFP; after puromycin selection 90–100% of cells expressed GFP. With this retroviral vector system no baseline expression of GFP was observed in the genetically modified IMKc. Dox treatment of these transduced cells induced GFP expression in a dose- and time-dependent manner. Peak GFP expression occurred after 72 h of Dox treatment and dropped to baseline when Dox was removed. Our observations demonstrate an efficient method for achieving stable Dox-regulatable transgene expression in human keratinocytes. These transduced keratinocytes maintain their ability to form a functional epidermis *in vitro*.

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The Secret of Measuring Sweat pH: With the Incorporation of Important Controls, there are no Gender Differences in Axillary Sweat pH Values

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As the pH of eccrine sweat is a critical factor for the gelation of antiperspirant actives, the accurate measurement of axillary sweat pH is of vital importance for the antiperspirant industry. Although one study has reported that females have an axillary sweat pH of 7.0 and males a sweat pH of 5.6, three other studies have demonstrated that no gender differences exist in axillary skin surface pH. In order to clarify this situation, we have executed a series of studies aimed at measuring the pH of thermally induced axillary sweat with the incorporation of critical controls; the most important of these being the equilibration of sweat samples with physiological levels of CO_2. In the present study, two age-balanced groups of male and female subjects (18–60 years) were recruited with the requirement that they had refrained from using antiperspirant products in the axilla for a period of at least 5 weeks. Three days prior to the study, subjects shaved their test axilla. On the study day, sweating was induced at a temperature of 43 ± 1°C and 65 ± 5% relative humidity. Following a warm-up period, eccrine sweat droplets were collected from the axillary vault and placed in glass vials. In order to mimic physiological conditions and thus gain a true representation of sweat pH, pH was measured following equilibration of samples with 5%CO_2/95%O_2; the rationale for this being that sweat pH is primarily controlled by a HCO_3^-/CO_2 buffer system and uncontrolled losses of CO_2 to the atmosphere leads to artificially high pH readings. Using this methodology, we demonstrated that the pH of axillary sweat was 6.54 ± 0.32 (mean ± standard deviation, n = 18) for the male group and 6.50 ± 0.31 (n = 15) for the female group. These readings were not significantly different (p = 0.74; unpaired t test).

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Assessing the Irritation Potential of Antiperspirants

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Adverse reactions from cosmetics are rare relative to their widespread and regular usage. However, both irritant and allergic contact dermatitis from cosmetics is well established, and underarm products have been implicated in causing a significant proportion of these reactions. In order to measure the irritation potential of marketed underarm products, and to enable the development of less irritating antiperspirants, we have developed a range of sensitive and reproducible measures of the irritation potential of this class of cosmetic. The single application forearm patch test is an appropriate rapid screen for irritation potential that is shown to discriminate between marketed antiperspirants. Including a model treatment for underarm shaving has further enhanced the relevance of the forearm patch test methodology. This modification is shown to increase the observed irritation potential of underarm systems –, e.g. in a 50 panellist test the frequency of occurrence of visible inflammation changes from 21 out of 50 (unshaved patch) to 35 out of 50 (shaved patch) for the no treatment control site. Product rankings, however, are not affected. Finally, an exaggerated-use axillary protocol (comprising a direct paired-comparison of two products over a four-week period) has been developed to assess irritation potential under more consumer relevant conditions. This less rapid, more resource intensive protocol, is used to validate the results from the forearm patch test screens. These clinical protocols have been used to assess the irritation potential of a range of marketed antiperspirants, and have highlighted an exceptionally mild antiperspirant stick. The comparison of this stick with a standard marketed stick in a 31-panellist exaggerated-use axillary test showed it to be consistently and significantly milder with respect to expert assessed irritation. The ultramild stick was assessed as milder in 26 panellists, harsher in 3 panellists and not different in 2 panellists during the final test week ($p < 0.001$).

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Sub-Surface Skin Imaging and Evaluation by Optical Coherence Tomography (OCT)

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Optical Coherence Tomography (OCT) is an emerging powerful imaging technique for probing turbid tissue like skin below surface in a noninvasive manner. It works similar to high-frequency ultrasound imaging except of the employment of near-infrared light rather than acoustical waves. The benefits compared to ultrasound imaging are considerably higher spatial resolution and the optional evaluation of tissue via optical parameters. Potential applications for OCT are in cosmetic/pharmaceutical research and clinical diagnosis as well. Particularly in cosmetics, repeated and rapid skin testing is required during product development to ensure efficacy and to monitor general skin reactions. In clinical routine, various types of skin diseases may be diagnosed more rapidly and cost-efficient as compared to biopsies and subsequent histologies. We assembled an OCT device to perform imaging in 2 (2D) and 3 (3D) dimensions at a resolution of about 5 μm (cell size). Penetration depths of up to 1 mm can be achieved, depending on the type of tissue. An additional feature of the device is to evaluate local moisture content of tissue. Skin 2D and 3D data sets from volunteers have been acquired in a research study. Morphological features in stratum corneum, epidermis and upper dermis can be delineated. Dynamic processes of externally applied perturbations have been monitored over a duration of 1 h. In a preliminary medical study NCN (Naevus Cell Naevus) and adjacent healthy skin are compared with histology. Relative local moisture levels were retrieved by additional data processing. Comparing to histology, our OCT images are in many cases of sufficient quality to serve as prediagnostic tool at penetration depths between 0.5 and 1 mm. Relative local moisture content can only be retrieved by OCT under noninvasive conditions.

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Epidermal and Dermal Dimensions of Human Skin can be Determined *In Vivo* by Optical Coherence Tomography

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Optical Coherence Tomography (OCT) is a new technique to record skin structures at various depths. This fills the gap between the high resolution (~ 1 micron) Confocal microscopy and lower resolution techniques like ultrasonography and Magnetic Resonance Imaging (resolutions 50–300 microns). OCT images skin as vertical sections with resolutions reaching 3 microns in lateral direction and 5 microns in depth. OCT is based on an optical interferometry principle where a broad band light beam of short coherence length is split into sample and reference beams; the reflected light is coupled to produce an interference signal. Dermal and epidermal structures are clearly outlined. Stratum corneum (SC) is visualized as a bright band and its thickness can be estimated. The geometry of isolated legions such as comedones and seborrheic keratosis can be visualized. OCT is useful to follow changes induced by experimental chemicals including irritants. OCT studies are at a very early stage but offer great promise as a means of studying human skin in health and disease.

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Immunoelectron Microscopic Analysis of Lysosomal Deposits in α-N-acetylgalactosaminidase (α-NAGA) Deficiency with Angiokeratoma Corporis Diffusum (ACD)

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α-NAGA deficiency with ACD (Kanzaki disease) is one of the lysosomal storage diseases. Since the substrate of α-NGAG is GalNAcα1-*O*-Ser/Thr, this substance is theoretically accumulated in lysosomes, but galactose and/or neuraminic acid conjugated substances, typically NeuAcα2–3Galβ(NeuAcα2–6) 1–3GalNacá1-*O*-Ser/Thr, are actually excreted in patient urine. In this study, we analyzed the lysosomal deposits in two Japanese patients by immunoelectron microscopy with mouse monoclonal antibodies to Tn, sialosyl Tn and T antigens to know whether GalNAcα1-*O*-Ser/Thr, which is a Tn antigen, or other materials are deposited in patients lysosomes. We stained ultrathin sections of specimens routinely fixed with osmium tetroxide and embedded in epoxy resin after a bleaching procedure and obtained excellent results from morphorogical and immunohistochemical point of view by this simple method. The results of immunoelectron stainings were that only Tn antigen was positive and we found that GalNAcα1-*O*-Ser/Thr was accumulated in enlarged lysosomes of vascular endothelial cells, eccrine sweat gland cells and fibroblasts of patient's skin. These results clarified that conjugation of galactose and neuraminic acid to this substance does not occur in any cells of the skin. These sugar conjugation appears to be carried out in other cells or organs and this suggests that this disease is caused by a deficiency of a single lysosomal enzyme.

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Innervation Changes of the Vulval Vestibule in Patients with Vulvodynia

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Vulvodynia is a condition characterised by the sudden onset of a painful burning sensation, lowered pain thresholds (hyperalgesia), mechanical allodynia, and occasionally pruritus, of the vaginal vestibule. Pain precipitated in the absence of nociceptor stimulus might be triggered by the release of mediators that set off inappropriate impulses in nonmyelinated pain fibres, thus sensitising the dorsal horn neurones. The objective of this study is to demonstrate the pattern of the innervation of the vulval vestibule, and distinguish any variance from this normal pattern, in vulvodynia patients, which might provide objective evidence for a sensory neurone abnormality. Biopsies were obtained from the affected area of patients ($n = 10$), and from a matched site of asymptomatic controls ($n = 8$), and processed for immunocytochemistry. The mean age of patients and controls was 32 years, and patients, satisfied Friederich's criteria for vulvodynia. 15 μm frozen sections were immunostained with antisera to the neuropeptide calcitonin gene related (CGRP), and the general neuronal marker PGP9,5, using the peroxydase avidin-biotin complex protocol (ABC). Sections were studied by computerised image analysis (Image Pro-Plus). The results reflect the mean of visual estimates of the density of immunoreactive innervation in the epidermis, and papillary dermis, where the pattern of the innervation presented quantitative variability between patients and controls. The quantitative grading was performed indipendently by two observers who were unaware of the study group to which each of the specimens belonged. The overall pattern of the distribution of the immunoreactive axons was similar in patients and controls qualitatively. However, vulvodynia patients specimens, had significant statistical increase in the number of PGP9,5 immunoreactive fibers in the papillary dermis (up to 62%) and in the epidermis (up to 30%), compared with the controls. Furthermore, considerable number of the PGP9,5 immunoreactive nerve fibres enters or run along and through the epidermis of affected subjects. Interestingly, no significant change was found in the distribution and discrete quantitative analysis of the CGRP immunoreactive axons between patients and controls. This increase in the sensory innervation of the papillary dermis and epidermis of the affected areas may be of diagnostic and aetiologic significance in vulvodynia.

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Familial Cylindromatosis: A Mutation in the CYLD Gene Undelies Multiple Tumors of Skin Appendages and Allows Prognostic Diagnosis for Genetic Health Services

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Familial cylindromatosis (turban tumor syndrome; Brooke–Spiegler-syndrome) (OMIM numbers 123850; 313100) is a rare skin disorder, inherited in an autosomal dominant fashion and usually presenting in the second or third decade of life. With female preponderance, dermal tumors predominantly arise in hairy areas of the body with approximately 90% on the head and neck, rarely on the face or trunk. Transformation to malignancy seems to be rare. Recently, the susceptibility gene CYLD which reveals the genetic attributes of a tumor-suppressor gene (recessive oncogene) has been localized to chromosome 16q and mutations have been identified in affected families. Here, we studied the molecular basis of familial cylindromatosis in a multigeneration family of German origin. Using PCR, microsatellite analysis, heteroduplex analysis, and automated sequencing, we identified a frameshift mutation in the CYLD gene in several individuals. Mutation analysis most importantly allowed exclusion of the tumor predisposition in a 6-year-old boy. Interestingly, in several family members with identical genotype we observed distinct phenotypic expression. While some individuals revealed discrete small skin colored tumors localized in the nasolabial region, one family member showed expansion of multiple big tumors on the trunk and in a turban like fashion on the head. The reasons for different clinical expression patterns of the same genetic defect in this disease remain elusive. Identification of the CYLD gene and modern genetic techniques enable us to rapidly confirm putative diagnoses on the genetic level, to give a prognosis for family members at risk for developing multiple tumors, and to provide affected families with genetic counselling.

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HLA-Haplotypes in Patients with Porphyria Cutanea Tarda and their Relatives

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HLA-haplotypes distribution has been studies in 50 patients with porphyria cutanea tarda (PCT) (42 men and 8 women) and their relatives (30). The clear manifestation of the disease was not observed in 44 patients, but these patients did have dyschromia, atrophic scar, and hypertrichosis of the facial skin. The vulnerability cutis of the facve and hands was noted in all the patients. The haplotypes A1B8, A1B18, A3B7, A2B5 were revealed during investigation. But the haplotype A1B18 was occurred the most frequently ($p < 0.05$). The haplotypes A1B8 and A3B7 are markers of the skin damage. The haplotype A1B18 was found in patients with liver disease. The examination of the patients' relatives showed the high rates of antigen. A2, B12, B13, Dr1 and Dr5. The haplotypes A2B12 and A2B13 were associated with hypertrichosis and hyperpigmentation symptoms. The increased rate of antigen B18 occurring with combination of antigen A1 confirms about linkage equilibrium between these antigens. It may be used in popular genetic to prognoses the risk of disease development.

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Update: A Population-Based Genetic Epidemiologic Survey of Alopecia Areata

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We are examining a broad AA phenotype by collecting families and genotyping candidate genes. Candidates were largely self-referred through information made available from the National Alopecia Areata Foundation and a project website. In addition, subjects were identified from the Dermatology clinics at the New York Presbyterian Hospital/Weill Medical College of Cornell University. Of 316 AA patients, 292 (92.4%) consented to participate. The racial composition of all consenting participants ($N = 292$) is 11% African-American, 3% Asian-American, 72% Caucasian, 13% Hispanic, and 1% Other. The racial composition of probands with a family history ($N = 124$) is 6% African-American, 82% Caucasian, 10% Hispanic, and 2% Other. Age-at-onset of the probands ranged from 9 months to 70-year-old and sibship size ranged from 0 to 9. Of the 124 probands with a family history of AA, 56% ($N = 69$) have a first-degree relative with AA, 19% ($N = 23$) have a second-degree relative with AA, and 27% ($N = 34$) have a third-degree relative with AA. DNA from blood samples of probands with ($N = 25$) and without ($N = 33$) a family history of AA was typed for polymorphisms at the HLA-D locus. Of the 58 patients, 21% were DR4 positive. Fourteen of the 58 (24%) probands who were HLA typed carried the DQA1*0501-DQB*0301 haplotype. These interim data suggest that AA aggregates in families. We plan to perform candidate gene analysis and genome scanning of AA in multiplex families from this cohort to facilitate the identification of disease susceptibility genes.

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Excessive Facial Hair is Common in Both General and Physician Office Populations

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Unwanted facial hair is a common problem for women. However, the prevalence of excessive facial hair in patients seen in physicians' offices, a population with good potential for treatment, is unknown. The aim of this study was to measure the prevalence of excessive facial hair in women seen in physicians' offices. A convenience sample of 200 women was surveyed for excessive facial hair in physician offices: 100 in a dermatology office and 100 in a family physician practice. Another 100 were surveyed outside of medical offices for comparison (84 in a shopping mall plus 16 at a health fair). Subject demographics, hair removal techniques, menstrual history, other medical problems, and quality of life were assessed. A modified Ferriman-Gallwey (F-G) scale was used as an objective self-assessment of facial hair. Overall, 26% of subjects reported excessive facial hair: 16% in the dermatology office, 29% in the family physician office, and 32% in the nonmedical setting ($p = 0.02$, c2 test of association). Excessive facial hair was more common in non-Whites (32%) than in Whites (23%), but the difference was not statistically significant. Excessive facial hair was positively associated (two-sided $p < 0.05$) with obesity, extremes of menstrual flow, number of hair removal methods, and the F-G score, which confirm the construct validity of the measure. In conclusion, excessive facial hair is common in both the general population and the populations seen in dermatologic and family physician offices. Physicians and the public should be made aware of currently available treatment options.

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Lupus Erythematosus Autoantibodies among Patients without Collagenosis Symptoms

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The study was carried out on sera of 130 consecutive patients who sought medical advice during a prophylactic action (65 females and 65 males, aged 9–82 years). None of the patients was suspected of having autoimmune disease. The serum samples were tested using EIA (Varelisa ReCombi ANA, Pharmacia & Upjohn) for the presence of autoantibodies specific to the following antigens: dsDNA, RNP, and Sm. Presence of these antibodies is considered typical for SLE. One or more antinuclear autoantibodies were detected in 18 of all 130 patients; 13 of 65 females were ANA-positive (13/65F, 20.0%) in comparison to 5 of 65 males (5/65 M, 7.7%). The dsDNA-specific autoantibodies were found in 8/65F and 4/65 M (total 12/130), RNP – in 4/65F and 2/65 M (total 6/130), Sm – in 2/65F and 0/65 M (total 2/130). In general, ANAs were more frequently detected in females ($p = 0.05$), however, differences for each particular antibody did not prove statistically significant. Altogether, antinuclear autoantibodies were found in 13.8% of subjects with no symptoms of autoimmune disease (confidence interval at $p = 0.05$: 7.9–19.8%). These figures should be kept in mind when interpreting results of tests for ANAs. To our knowledge, this is the first serological survey on antinuclear autoantibodies among Poles free from autoimmune disease.

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The Prevalence and Clinical Significance of Anti-U1 RNA Antibodies in Patients with Systemic Sclerosis

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We studied the prevalence and clinical significance of anti-U1 RNA antibodies (anti-U1 RNA) in patients with systemic sclerosis (SSc). The presence of anti-U1 RNA was determined using immunoprecipitation in SSc patients with anti-U1 RNP antibodies (anti-U1 RNP) ($n = 36$), antitopoisomerase I antibodies ($n = 20$), anticentromere antibodies ($n = 20$), MCTD ($n = 23$), SLE patients with anti-U1 RNP ($n = 26$), and normal controls ($n = 20$). Moreover, antigen specificities for anti-U1 RNP were examined in patients with SSc by immunoblotting. Anti-U1 RNA was detected in 22 of 36 SSc patients (61%) with anti-U1 RNP, 14 of 23 patients (61%) with MCTD, and 8 of 26 SLE patients (31%) with anti-U1 RNP. Anti-U1 RNA was not detected in other groups. As for SSc patients, the frequencies of PF and reduced %DLco were significantly greater in patients with anti-U1 RNA than in those without (80% vs. 25%, $p < 0.005$; 86% vs. 45%, $p < 0.05$, respectively). Moreover, patients with anti-U1 RNA had significantly lower %DLco and %VC values than those without (47.1 ± 17.3 vs. 73.1 ± 20.3, $p < 0.05$; 80.9 ± 21.2 vs. 96.0 ± 18.5, $p < 0.05$, respectively). Regarding the antigen specificities of anti-U1 RNP in SSc patients, the frequency of anti70-kDa was significantly lower in patients with anti-U1 RNA than in those without (45% vs. 86%, $p < 0.05$). These results suggest that anti-U1 RNA could be useful for the classification of SSc patients with anti-U1 RNP and serve as a serological marker for pulmonary fibrosis in those patients.

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Discoid Lupus Erythematosus (DLE)-Like Lesion Induced by Uracil Tegafur (UFT)

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A mixture of uracil and tegafur (UFT) is a common antineoplastc agent in Japan. Fluorouracil agents include tegafur, fluorouracil and UFT. The keratotic vesicular pigmented type accounts for 45% of cases with fluorouracil agent-induced skin lesions and discoid lupus erythematosus (DLE) type for about 10%. First, we report a 64-year-old Japanese woman with DLE-like lesions which were induced by UFT. After surgery to treat lung cancer, UFT (300 mg per day) was administered and she developed round erythema at her right cheek. A skin biopsy specimen revealed atrophy of the epidermis, a slight liquefaction, and patchy lymphocytic infiltration. Antinuclear antibody was weakly positive. After discontinuation of UFT, the erythema completely regressed within 2 months. Second, we collected 17 patients of DLE-like lesions induced by fluorouracil agents from case reports in Japan and summarized the common features. Among the 3 different fluorouracil agents, UFT showed the highest frequency of DLE-like eruptions (18%). When compared with 31 cases of idiopathic DLE which we experienced, the positive ANA ratio was relatively higher in fluorouracil agent-induced DLE-like eruption than in DLE. Despite the high ratio of ANA in the former cases, all of the ANA disappeared within 1 years after discontinuation of medicine. The positive LBT ratio was much higher in DLE than in fluorouracil agent-induced DLE-like eruption. Fluorouracil agent-induced DLE-like eruptions regressed within 2 months after discontinuation of medicine, whereas idiopathic DLE tends to persist for a long time. Fluorouracil agent-induced DLE-like eruption is an excellent model for better understanding the pathomechanisms of development of discoid lesions in LE, and further study may provide new insights into autoimmune interface dermatitis.

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Skin Findings in Proteus Syndrome

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Proteus syndrome is a sporadic, mosaic disorder with progressive asymmetric overgrowth of multiple tissues during childhood. These patients may have connective tissue nevi, linear epidermal nevi, vascular malformations, dysregulated adipose tissue, abnormal facial features, disproportionate overgrowth such as digital gigantism or hemihyperplasia, and tumors before age 20. We evaluated 23 consecutive patients with Proteus syndrome that meet published diagnostic criteria (Biesecker *et al* 1999). A total body skin examination was performed on 14 males and 9 females ages 10 months to 40 years (median age 12 years). The skin findings included connective tissue nevi in 20 patients (on the soles of the feet in 18), linear epidermal nevi in 15, cutaneous vascular malformations in 20 (3 capillary, 4 venous, 1 lymphatic, 10 capillary/venous, 1 capillary/lymphatic, and 1 capillary/venous/lymphatic malformation), lipomas in 20, and lipoatrophy in 9. Dermal hypoplasia was present in 2 of the 23 patients. The extent of these skin findings varied from small 3-mm macules and papules to massive lesions distributed over the trunk and extremities. More extensive skin findings appeared to correlate with more severe overgrowth of bones and internal organs. Features of Proteus syndrome not previously reported include connective tissue nevi on the palms in 3 and accessory nasal alae in 2 patients. Although it is a mosaic disorder that is variable in extent and areas of involvement, Proteus syndrome exhibits a defined spectrum of cutaneous manifestations.

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Prevalence of HIV-Associated Opportunistic Skin Diseases under Highly Active Antiretroviral Therapy

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Highly active antiretroviral therapy (HAART) strongly reduced incidence and severity of numerous opportunistic diseases in patients with AIDS and significantly improved life expectance. The broad documentation of these findings did not allow to determine the relation between clinical improvement and restoration of immune functions. The strong reduction in opportunistic diseases may be due either to the reconstitution of the immune system or to the direct antimicrobial effects of substance such as proteinase inhibitors. To assess the clinical significance of the immune reconstitution in HAART-treated patients, we compared prevalence and severity of HIV-associated opportunistic skin diseases in 910 HIV infected patients in two cohorts that were normalized to comparable numbers of CD4+ T cells. Cohort I had not received HAART, cohort II was treated with HAART. Mantel-Haenszel test revealed that HAART treated patients with CD4+ T cells < 200 per ml, significantly lower prevalence of oral candidiasis (0.56{0.33–0.71}) and Kaposi sarcoma (KS; 0.41{0.20–0.85}) than the control population. Surprisingly, the opposite was true for other uncomplicated zoster (2.01{1.34–3.01}) and viral warts (2.23{1.51–3.30}), which were significantly more prevalent in the HAART treated cohort II. Thus, HAART was a risk factor for the occurrence of viral warts and zoster, but directly protected against candidosis and KS. Importantly, protease inhibitors can directly abrogate the pathogenicity of various candida strains. Together the data suggest, that the percentage of CD4+ T cells in the peripheral blood of HAART treated patients overestimates the real immune reconstitution and that at least some benefits induced by HAART are related to the direct antimicrobial or anti-inflammatory effects of protease inhibitors.